Department of Production Animal Medicine
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Impact of pre-mating diet and metabolic state on follicle development and piglet birth weight in sows

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DOCTORAL DISSERTATION

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To my father…

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LIST OF ORGINAL PUBLICATIONS

This thesis is based on the following publications:

I. Han, T., Björkman, S., Soede, N.M., Oliviero, C., Peltoniemi, O.A.T., 2020. IGF-1 concentration patterns and their relationship with follicle development after weaning in young sows fed different pre-mating diets. Animal 14:1493-1501.

II. Han, T., Björkman, S., Soede, N.M., Oliviero, C., Peltoniemi, O.A.T., 2021. Pre-mating metabolic hormones are related to follicle development after weaning in primiparous sows fed dietary fibre before mating. Submitted.

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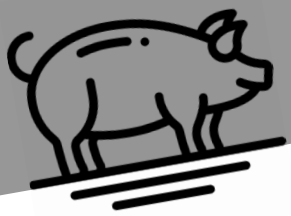
IV. Han, T., Björkman, S., Soede, N.M., Oliviero, C., Peltoniemi, O.A.T., 2021. Pre-mating metabolic hormones are related to litter characteristics at subsequent farrowing in primiparous sows fed dietary fibre before mating. Submitted.

CONTENTS



1. ABSTRACT

Piglet birth weight and within-litter birth weight variation are important for piglet survival and growth. Pre-mating diets and metabolic state of the sow may impact subsequent piglet birth weight. This is because pre-mating diets and metabolic state affect follicle and oocyte development, and thereby subsequent embryo and placental development, which largely determine piglet birth weight.

The objective of this thesis was to modulate the pre-mating metabolic state of young sows by using specific pre-mating diets supplemented with L-carnitine (LC), L-arginine (AR), microfibrillated cellulose (MFC) and sugar beet pulp (SBP). A further objective was to investigate if and how pre-mating metabolic state affects subsequent follicle development and piglet birth weight.

Sows received specific supplements to their pre-mating diets during the last week of lactation and weaning-to-oestrus interval (WEI) in both studies. In study I, a total of 56 first- and 20 second-parity sows received either wheat (CON) or wheat plus either MFC, LC or AR at one of two supplementation levels. In study II, 58 first-parity sows received treatment diets supplemented with SBP and MFC compared with commercial diets (CON). For both Study I and II, sows' body condition and its losses during lactation were assessed. From weaning till ovulation, follicle diameters were repeatedly measured with ultrasound. Pre-prandial blood samples were collected at weaning, at 3 days after weaning and at oestrus for metabolic hormones and metabolites analysis. At subsequent farrowing, sows after their first (N = 41) and second (N = 15) lactation in Study I and 44 sows after their first lactation in Study II were used for investigation of litter characteristics (litter size, piglet birth weight and piglet birth weight variation).

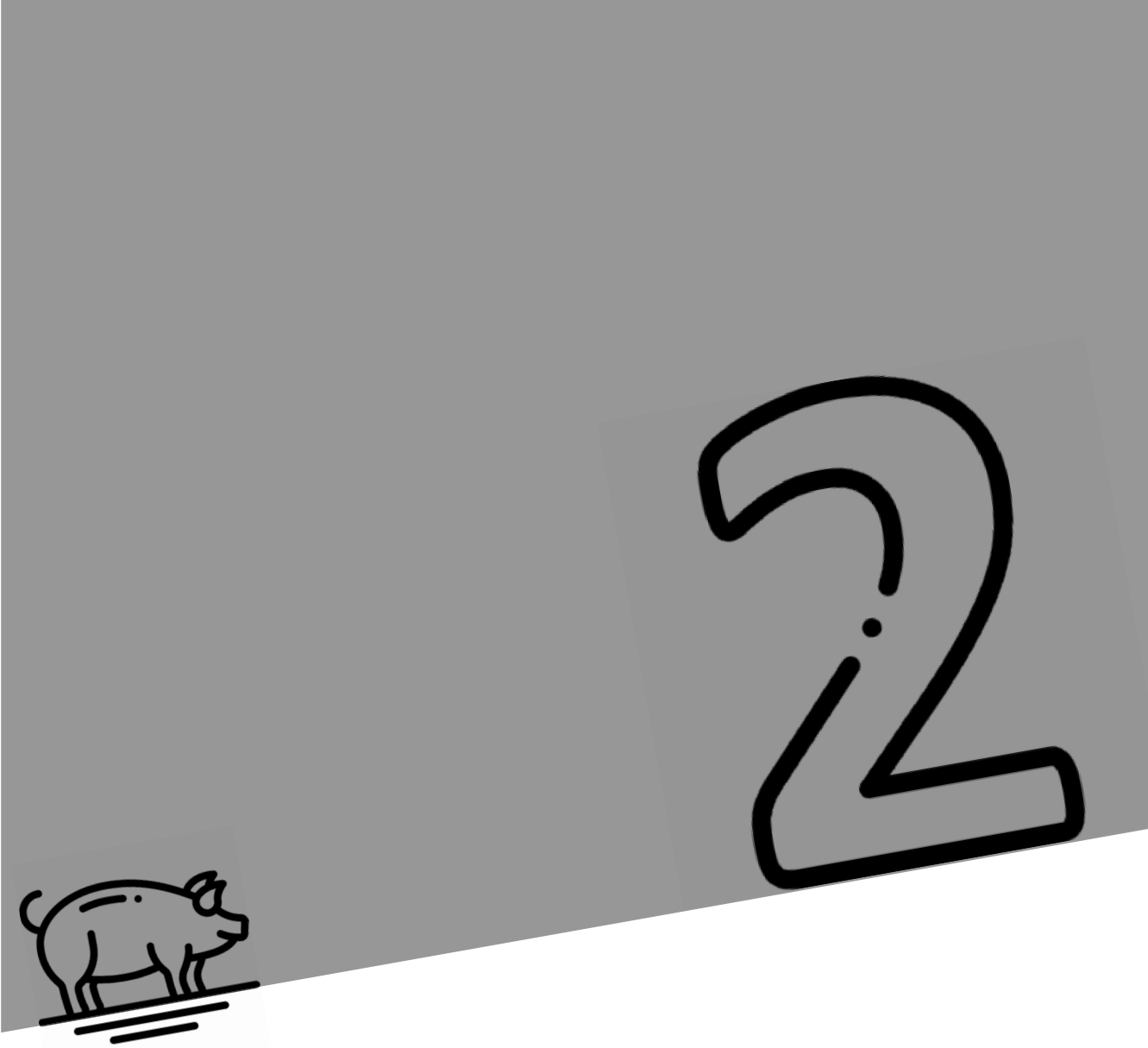
Before weaning, the pre-mating diets did not affect average daily feed intake (ADFI) and body condition losses in Study I. In Study II, however, SBP sows had higher backfat (BF) loss during the last week of lactation than MFC sows (1.1 v. 0.2 mm, $P < 0.05$). Higher creatinine concentration at weaning was observed in SBP sows than in MFC sows (163 v. 137 mmol/L, $P < 0.001$). However, the pre-mating diets did not affect insulin-like growth factor-1 (IGF-1) concentration, follicle development or litter characteristics at subsequent farrowing in either studies.

At weaning, sows with higher plasma IGF-1 had larger follicles ($\beta \geq 0.001$, $P < 0.01$ for both Study I and II). At 3 days after weaning, IGF-1 ($\beta \geq 0.002$, $P < 0.05$ for both Study I and II) and serum leptin

(β = 0.08, P < 0.05; Study II) concentrations were positively related to follicle diameters. Further, sows with higher IGF-1 concentrations at weaning and 3 days after weaning had shorter weaning-to-ovulation intervals (WOI; β ≤ -0.03, P < 0.05 for both).

At subsequent farrowing, IGF-1 at weaning after first lactation was positively (β ≥ 0.01, P < 0.06 for both studies) and creatinine concentration at weaning in Study II was negatively (β = -0.1, P < 0.001) related to litter weight. Higher IGF-1 concentration at 3 days after weaning and at oestrus after first lactation were related to larger mean piglet birth weight at subsequent farrowing in Study I (β ≥ 1.0, P < 0.05 for both). Lower non-esterified fatty acid (NEFA) concentration at oestrus was also related to CV of piglet birth weight in Study II (β = 28.8, P = 0.05). Body weight (BW) loss and BF loss were not related to litter characteristics. Surprisingly, in study I, a higher relative loin muscle depth (LM) loss (%) during the previous lactation in both parity sows resulted in more uniform piglets (lower CV and SD of birth weight, P < 0.05). In Study II, higher LM loss during the last week of the previous lactation was related to higher proportions of small piglets (β = 0.9, P < 0.05) and lower litter weight (β = -0.5, P = 0.02) at subsequent farrowing.

In conclusion, the supplemented compounds in the pre-mating diets of young sows did not improve follicle development after weaning or litter characteristics at subsequent farrowing. However, changes in metabolic hormones and metabolites related to sows' body condition loss during lactation were associated with follicle development after weaning and litter characteristics at subsequent farrowing. Plasma IGF-1 positively affected both follicle size and piglet birth weight. However, the positive impact of IGF-1 on piglet birth weight differed based on the sows' negative energy balance (NEB) during lactation. Plasma IGF-1 concentration before weaning (i.e. during lactation) in sows with mild NEB and IGF-1 around the time of oestrus in sows with severe NEB both positively affected subsequent piglet birth weight. These results suggest that higher plasma IGF-1 concentrations during lactation and after weaning, resulting from minimizing sows' body condition losses during lactation, will not only improve follicle development after weaning but will also improve litter characteristics at subsequent farrowing in young sows.



2. INTRODUCTION AND LITERATURE REVIEW

2.1. Introduction

The first domesticated pigs appeared 10 000 years ago (Larson et al., 2011). During domestication, pig production systems became more intense which resulted in more stress to pigs and negative impacts on animal welfare (reviewed by Albernaz-Gonçalves, et al., 2021). In the future, more focus on animal welfare is likely needed in order to mitigate the effects of the rapidly changing world and increase resilience of this production animal species (Peltoniemi et al., 2021a). However, genetic selection for larger litter size still goes on. Recent hyper-prolific sows farrow around 16-20 piglets. For instance, the litter size of Danish and Dutch breeds in recent studies was on average 19.8 (Kobek-Kjeldager et al., 2020) and 16.3 (Costermans et al., 2020a), respectively. This increased litter size resulted in a higher pre-weaning mortality rate (Wientjes et al., 2012a). Pre-weaning mortality ranging from 13% to 27% have been reported in large litters (litter size ranged 16-20; Wientjes et al., 2012a; van den Bosch et al., 2019; Kobek-Kjeldager et al., 2020). One of the most important factors affecting pre-weaning mortality in large litters is increased within-litter piglet birth weight variation (Peltoniemi et al., 2021b).

Piglet birth weight characteristics (i.e. birth weight and its variation within the litter) are important for their survival and growth. Numerous studies have shown that piglets with low birth weight have higher pre-weaning mortality (Baxter et al., 2008; Muns et al, 2013; Hawe et al., 2020; Kobek-Kjeldager et al., 2020). This is because they are more vulnerable due to impaired thermoregulation and low energy body stores. This results into low viability and capability to compete for colostrum with their littermates (Rutherford et al., 2013). Feldpausch et al. (2019) showed that one-third of piglets die during lactation if their body weight was below 1.11 kg, and this mortality represents 43% of total pre-weaning mortality. Also, piglets below 1 kg of birth weight had higher pre-weaning mortality (20.9%) and they died on average at 9.2 days of lactation with 1.2 kg of body weight (BW; Hawe et al., 2020). Not only individual birth weight but also its variation is connected to higher pre-weaning mortality. This is because piglet birth weight variation (litter uniformity) is related to proportions of small piglets. For instance, when the CV of birth weight increased by 1%, the proportions of low birth weight piglets (< 800 g) increased by

1.31% and pre-weaning mortality increased by 1.08% (Wientjes et al., 2012a). Even if small piglets survive, they have an impaired average daily gain and therefore higher slaughter age (Rehfeldt et al., 2008; Beaulieu et al., 2010; Smit et al., 2013). Thus, increasing piglet birth weight and decreasing within-litter piglet birth weight variation are very important for improving piglet survival and growth rate, thereafter affecting both pig farm productivity and animal welfare.

One of the factors affecting piglet birth is the body condition loss of the sow during previous lactation (Wientjes et al., 2013a). Body condition loss during lactation influences pre-mating metabolic state, and thereby affects follicle development, which may affect litter characteristics at subsequent farrowing (reviewed by Prunier and Quesnel, 2000). Around 10-12% of BW loss during lactation can be considered indicative of severe negative energy balance (NEB) and is associated with reduced reproductive parameters, such as prolonged weaning-to-service interval, lower farrowing rate and decreased litter size (Thaker and Bilkei, 2005). These negative effects are caused by impaired follicle and oocyte development after weaning (Zak et al., 1997; Costermans et al., 2020a), embryo weight and survival (Patterson et al., 2011; Hoving et al., 2012), and finally piglet birth weight (Wientjes et al., 2013a). These effects are even more severe in large litters. Hyper-prolific sows have high metabolic demands for milk production for their large litters, which results in high body condition losses during lactation (Strathe et al., 2017). Especially younger parity sows with a low feed intake capacity during lactation (Strathe et al., 2017), easily experience severe NEB during lactation. Thus, younger sows are at higher risk of having the impaired fertility. This needs to be prevented and it is therefore important to understand where piglet birth weight derives from and how pre-mating metabolic state affects piglet birth weight.

The literature review starts with a discussion on how follicle development affects piglet birth weight (Paragraph 2.2). After that, the physiology of follicle development is explained in detail (Paragraph 2.3), followed by a description of body condition changes and metabolic hormones during lactation (Paragraph 2.4). Also, how these metabolic changes and hormones affect the physiological process from follicle development to piglet birth weight is discussed (Paragraph 2.5). A possible approach to improve sows' metabolic state before breeding is modulating dietary composition. The composition of the pre-mating diet impact on both follicle development and piglet characteristics at subsequent farrowing (reviewed by Campos et al., 2012; Peltoniemi et al., 2021b). Therefore, different dietary composition are discussed in paragraph 2.6.

2.2. From follicle development to piglet birth weight

For preventing small piglets at birth and thereby reducing the incidence of piglet mortality during lactation, it is important to understand where small piglets derive from. Firstly, genetic selection for litter size has led to an increase in ovulation rate. Increased ovulation rate results in insufficient uterine space for the placenta, which impairs embryo and foetal development and growth. For instance, sows which had one ovary and one uterine horn surgically removed (i.e. unilateral-ovary-hysterectomy (UOH)) had less uterine space compared to sows with unilateral oviduct ligation. Further, sows with UOH had a similar ovulation rate compared to intact sows because of compensatory ovarian hypertrophy with one uterine horn. Thus, the decreased uterine space per embryo results in increased uterine crowding in sows with UOH (Pére et al., 1997; Pére and Etienne, 2000; Vallet et al., 2014). The result of this was sows with UOH had a higher number of embryos in both days 35 of pregnancy but a lower number and weight of foetuses and placentae at days 112 of pregnancy. The results indicate that uterine crowding results in higher pre-natal mortality and lower pre-natal foetal weight. Similarly, in intact sows, a higher ovulation rate caused lower implantation length at days 35 of pregnancy in sows (Da Silva et al., 2016). This lower implantation length develops into smaller placenta (Stroband and van der Lende, 1990), which has limited ability to transport nutrient and blood from the sow to the foetus (Wootton et al., 1977; Finch et al., 2004). Thus, increased ovulation rate results in smaller embryos around the time of implantation due to uterine crowding, thereafter reducing foetal and placental size, which lead to piglets with smaller birth weight.

In addition to increased ovulation rate and subsequent uterine crowding, also impaired follicle development affects embryonic, foetal and placental development and subsequent piglet birth weight. The follicular fluid contains steroids, produced by granulosa and theca cells, and nutrient and hormones from the blood, which are crucial for oocyte maturation before ovulation (Dumesic et al., 2015). At weaning, during the early-follicular phase, smaller follicles contain lower oestradiol concentrations in their follicular fluid (Costermans et al., 2020b). This indicates that small follicles may be behind in the development because the initiation of oestradiol production in follicles is the key factor for their further development (Foxcroft and Hunter, 1985). During the mid-follicular phase, smaller follicles had lower follicular fluid insulin-like growth factor-1 concentrations (IGF-1) and COCs expansion during in vitro maturation (IVM; Costermans et al., 2020a). In addition,

positive relations were found between follicular fluid volume and oestradiol concentrations at the mid-follicular phase (Costermans et al., 2019a). Also in the late-follicular phase, before the luteinizing hormone (LH) surge, smaller follicles had lower oestradiol in follicular fluid (Zak et al., 1997; Yang et al., 2000). Therefore, small follicles have a poor microenvironment for oocyte development.

Between ovulation and implantation, oocytes from small follicles are connected to impaired embryo development (i.e. less developed or low survival). A lower percentage of blastocyst after in vitro fertilization (IVF) were found in fertilized oocytes from small follicles (Yoon et al., 2000; Marchal et al., 2002; Bagg et al., 2007). In addition, fertilized oocytes from small (< 3 mm) follicles developed into less developed blastocyst at 8 days after IVF compared to fertilized oocytes from large (≥ 3 mm) follicles. This is because oocytes from small follicles store less mRNA or protein, which regulate fertilization and embryo development before implantation (De Sousa et al., 1998), and have less glycolytic activity during maturation (Krisher, 2004). Impaired embryos will not elongate as much as more developed embryos because elongation is dependent on the individual developmental stage of ovoid morphology (Geisert et al., 1982). Also, those less developed (not fully elongated) blastocysts hardly attach to the uterus because more developed embryos secrete oestradiol around days 10-11 of pregnancy, which negatively affects less developed embryos' elongation and implantation (Waclawik et al., 2017). Even if less developed embryos attach to the uterus, their implantation sites will be smaller than those of more developed embryos, as previously suggested by Wrathall (1971). This small implantation site makes the situation worse since a small implantation site and placenta cannot fully provide the nutrients and blood. After implantation, small foetuses have small placentae (Kight et al., 1977; Vallet et al., 2014) and the weight of these foetuses and their placentae remain small throughout pregnancy. Eventually, the impaired placental function results in small birth weight piglets (Knight et al., 1977). Thus, the relations between small follicles and impaired oocytes before ovulation seem to cause less developed embryos, smaller foetal and placental development, therefore being associated with small birth weight piglets. Furthermore, in large litters, both small and compromised follicle before ovulation, and uterine crowding result in a higher chance of increasing the number of small piglets, as described in Fig. 2.1.

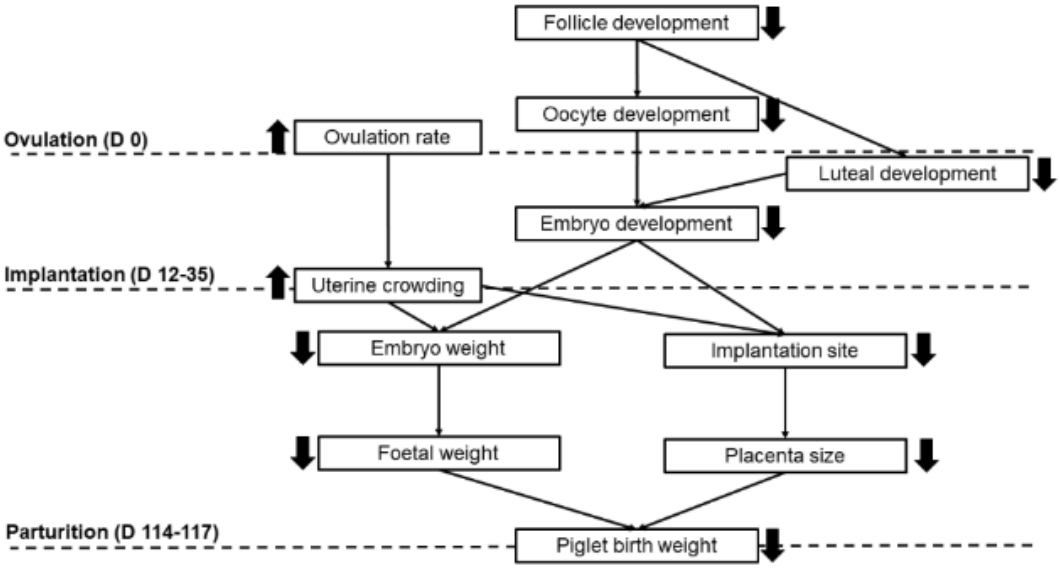


Fig. 2.1. Schematic illustration of the possible pathway from follicle development to piglet birth weight. Solid arrows indicate potential consequences of previous parameters on subsequent parameters reported previously.

2.3. Follicle development in sows

During lactation, the suckling behaviour of piglets suppresses LH secretion via inhibiting the gonadotropin-releasing hormone (GnRH)-pulse generator in sows (Britt et al., 1985). This low LH results in small follicle diameters during lactation. As lactation proceeds, LH-pulsatility and concentration are normally restored (van den Brand et al., 2000), and thereby follicle diameters increase (Britt et al., 1985; Fig. 2.2). Palmer et al. (1965) noted that the size of larger follicles increases, while the number of smaller follicles decreases over the course of lactation. A recent study also showed that follicle diameters during lactation increased and sows with larger follicles at the beginning of lactation had larger follicle diameters at the end of lactation (Lopes et al., 2020).

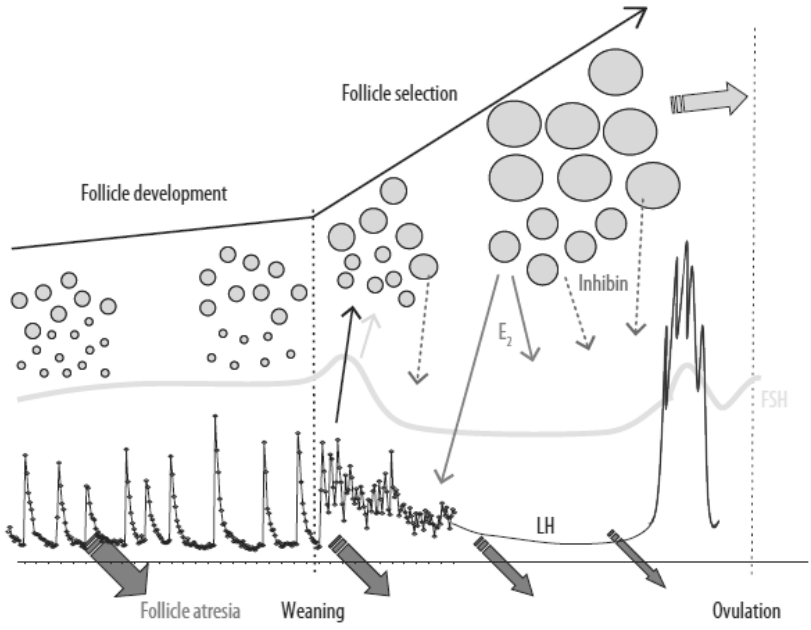


Fig. 2.2. Regulation of follicular development in late lactation and weaned sows, showing the most relevant reproductive hormones (Soede and Kemp, 2015).

After weaning (i.e. start of the follicular phase), the largest antral follicles are recruited and initiate to develop. Recruited follicles may still suffer atresia during the follicular phase, while selected follicles become pre-ovulatory follicles (7-8 mm) and ovulate (reviewed by Guthrie, 2005; Knox, 2005). The GnRH/LH secretion pattern plays a major role in the recruitment of follicles from the pool. Changes of LH pulsatility from a lesser frequency/greater amplitude pattern to a greater frequency/lesser amplitude pattern is important for follicular recruitment and subsequent development (Fig. 2.2; Shaw and Foxcroft, 1985). During the follicular phase, FSH and LH affect follicle numbers and follicle growth (reviewed by Kemp et al., 1998; Knox, 2005). Luteinizing hormone stimulation will result in 17β-oestradiol (E2) and inhibin production by the larger follicles (Fig. 2.3A). This increased systemic E2 and inhibin exert negative feedback on the hypothalamus, resulting in decreased release of both FSH and LH (Fig. 2.3B). Because the inhibin specifically inhibits FSH release (Noguchi et al., 2010), FSH concentrations are reduced much more than LH concentrations. Smaller follicles have fewer LH-receptors and are more FSH-dependent, thus these follicles will go into atresia when FSH concentrations decrease (Lucy et al., 2001; Fig. 2.3B). The larger follicles are LH-dependent and will develop further, but some may still become atretic in the period before ovulation (Fig. 2.3C). In addition to the gonadotrophins FSH and LH, also metabolic hormones and metabolites, such as IGF-1, play a role in the selection and development of large follicles (this will be further discussed in paragraph 2.5).

Late follicular phase follicles produce increased levels of E2 (Prunier et al., 1987; Noguchi et al., 2010; Fig. 2.3A). These increased E2 concentrations induce the pre-ovulatory LH surge (Fig. 2.3B), which causes a rapid decrease of E2 (Fig. 2.3A), and luteinisation of follicles, which initiates progesterone production (Fig. 2.3A).

Follicle development during the follicular phase and timing of ovulation varies between sows and are affected by parity. Primiparous sows have smaller follicles at weaning (2.5 mm; Langendijk et al., 2000) because of relatively severe NEB during lactation than multiparous sows (3.3 mm; Gerritsen et al., 2008). Pre-ovulatory follicle diameter is usually 7-8 mm (average of the 3-5 largest follicles measured by ultrasonography), but this may vary among sows. Primiparous sows have somewhat smaller follicles at ovulation than multiparous sows (7 v. 8 mm; Langendijk et al., 2000; Gerritsen et al., 2008). Primiparous sows have also a longer time to oestrus and ovulation after weaning because small follicles need more time to become pre-ovulatory and produce E2 (Langendijk et al., 2000). Sows show oestrus around 3 h after E2 peak (Soede et al., 1994).

Ovulation occurs at a quite fixed time of 30 hours after LH peak (Soede et al., 1994). The duration of oestrus is on average 52 h and ovulation occurs on average 38 h after onset of oestrus (Soede et al., 1994).

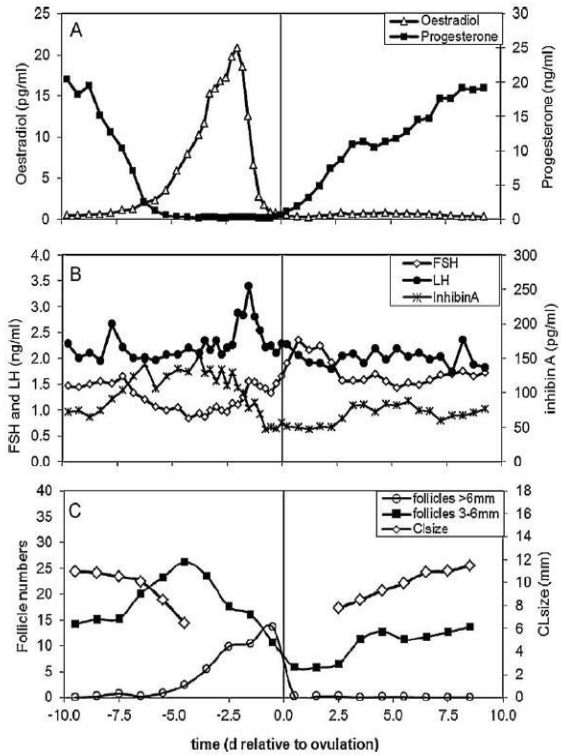


Fig. 2.3. Plasma concentrations of estradiol-17β and progesterone (A), and FSH, LH and Inhibin A (B), and follicular and CL development (C) during the oestrus cycle in sows (based on Noguchi et al., 2010). The vertical line in the figures represents the time of ovulation (Soede et al., 2011).

2.4. Sow's body condition losses during lactation and metabolic state

Body condition changes during lactation and related changes in metabolic hormones are connected to follicle development after weaning. During lactation, sows are in NEB because they have insufficient feed intake for the high metabolic demands for milk production and for maintenance (Bergsma et al., 2009), especially during the first two weeks (Feyera and Theil, 2017; Fig.2.4). The NEB results in mobilization of body reserves for energy production and therefore BW loss (Bergsma et al., 2009). Body weight loss consists of both fat and protein losses. Body weight and backfat thickness (BF) can be measured for estimating body fat mass and body protein mass (Whittermore and Yang, 1989), whereas measuring loin muscle depth (LM) can be also applied. These BW, BF and LM changes from farrowing to weaning are indicators of sows' body mobilization during lactation. During the last two weeks of lactation, sows get into positive energy balance as feed allowance reaches maximum (Feyera and Theil 2017; Fig. 2.4). However, this positive energy balance during 1 or 2 weeks is too short to compensate the body condition loss during lactation (reviewed by Eissen et al., 2000). Hyper-prolific sows may experience even more severe NEB during lactation. Higher milk production in hyper-prolific sows was connected to higher BW and BF losses during lactation ($r = 0.49$ and $r = 0.43$, $P \leq 0.001$ for both; Strathe et al., 2017). Primiparous sows also have more body condition losses because they have lower average daily feed intake (ADFI) (Strathe et al., 2017). For instance, primiparous sows lost 10-11% of BW (DanAvl, Strathe et al., 2017; TN70, Costermans et al., 2020a), while multiparous sows had 7-10% of BW loss during lactation (DanAvl; Strathe et al., 2017; Costermans et al., 2019b). Thus, younger sows are more vulnerable to losing their body condition during lactation and this can be represented by BW, BF and LM.

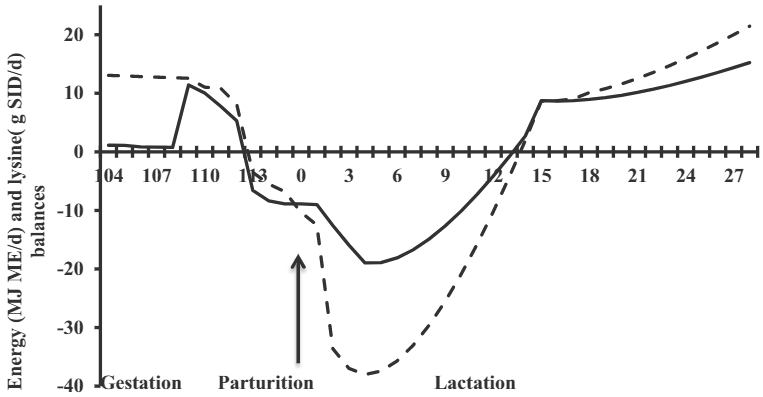


Fig. 2.4. Calculated metabolizable energy (ME; dashed line) and standard ileal digestible (SID) lysine (solid line) balances of the sow from late gestation to end of lactation (Feyera and Theil, 2017).

In addition to sows' body condition, also metabolic hormones and metabolites, such as plasma IGF-1, non-esterified fatty acid (NEFA), creatinine and leptin are known as indicators of the metabolic state of sows (Mosneir et al., 2010). Among these metabolic hormones, specifically IGF-1 concentrations before and after weaning are closely related to follicle development (will be discussed in paragraph 2.5). Insulin-like growth factor-1 secretion occurs in the liver and is affected by growth hormone (GH), feed intake and body condition losses. In early lactation, GH concentration is increased by the suckling stimulus by piglets and the increased feed intake of sows (Rushen et al., 1993; Fig. 2.5A). These together stimulate the liver to secrete and synthesize IGF-1 (van den Brand et al., 2001; Fig. 2.5A). During lactation, the IGF-1 concentration pattern is dependent on sows' feed intake but independent of GH concentration. This is because of the uncoupling between GH and IGF-1 during lactation (reviewed by Lucy, 2008). The uncoupling occurs when milk production reaches the maximum (Lucy, 2008), which is usually 16-18 days of lactation in hyper-prolific sows (Strathe et al., 2017). Therefore, sows have lower IGF-1 concentration at weaning than during lactation (van den Brand et al., 2001; Meija-Guadarrama et al., 2002; Wientjes et al., 2012b; Fig. 2.5B). Well-fed sows (mild NEB) have higher IGF-1 concentration at weaning than underfed sows (severe NEB; van den Brand et al., 2001; Mejia-Guadarrama et al., 2002; Costermans et al., 2020a; Fig. 2.5B). After weaning, IGF-1 concentration increases within 3 days (van den Brand et al., 2001; Wientjes et al., 2012b; Fig. 2.5B) or even earlier (Mejia-Guadarrama et al., 2002; Fig. 2.5B), probably due to increased insulin and changes

to the anabolic state after weaning. However, it remains unclear why sows restore their IGF-1 rapidly. These increased IGF-1 concentrations remain at this level until oestrus (Wientjes et al., 2012b; Fig. 2.5B).

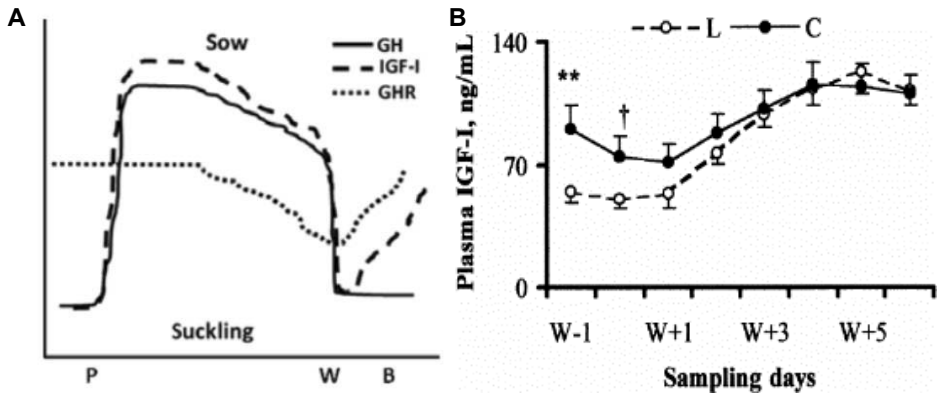


Fig. 2.5. Scheme of plasma growth hormone (GH), IGF-1 and liver GH receptor (GHR) during lactation (P: parturition; W: weaning; B: breeding) in sows (A; Lucy, 2008) and IGF-1 concentration after weaning (B; L: sows fed lysine restricted diet during lactation. C: control sows. Meija-Guadarrma et al., 2002).

As BW losses consist of both protein and lipid losses, metabolic parameters related to protein and lipid loss have been investigated by many researchers. Body fat mobilization in sows can be indicated by NEFA concentrations during lactation (Mosnier et al., 2010). None-esterified fatty acids and glycerol are produced by the degradation of triglycerides stored in adipose tissues when the animal is in NEB (Arner, 2003). Creatinine concentration is an indicator for protein mobilization as creatinine originates from the phosphorylation of creatine to provide energy in the muscle (Belstra et al., 1998). Accordingly, serum NEFA and creatinine concentrations are higher when sows mobilized their body lipid and protein, respectively. Concentrations of serum NEFA were positively related to BF loss (mm) during lactation ($\beta = 0.15$, $p = 0.03$; Costermans et al., 2019b; Table 2.1), which supports that measuring BF loss is an indicator for lipid mobilization. Higher serum creatinine concentrations were found around weaning when sows were fed a restricted feed level or restricted lysine content (Bidoo et al., 1992; Yang et al., 2009), which also supports that serum creatinine is an indicator of protein mobilization. Leptin is largely produced by adipose cells and reduces their appetite (Barb et al., 2001), and it was positively related to body fat mass and BF thickness (Prunier et al., 2001). Serum leptin concentrations in sows are higher during the

beginning of lactation, decreasing as the lactation period proceeds (Prunier et al., 2001; Costermans et al., 2020a), indicating that sows mobilized their body fat during lactation. After weaning, serum leptin concentrations increased within 2 days (Costermans et al., 2020a), showing that sow's fat reserves may be recovered after weaning. Thus, metabolic hormones during lactation may give a better reflection of metabolic state than body condition changes (Table 2.1). In addition, those metabolic hormones affect follicle and oocyte development which seem to be connected to piglet birth weight. Nevertheless, if and how these metabolic hormones before mating are associated with piglet birth weight at subsequent farrowing have not yet been investigated.

Table 2.1. Summary of production of metabolic hormones and metabolites and their relations to body condition in sows (discussed in paragraph 2.4)

Metabolic hormones and metabolites	Production site	Cause of production	Relations to body condition
IGF-1	Liver	Stimulation of GH	Loss -
NEFA	Adipose tissue	Degradation of triglycerides	Loss +
Creatinine	Muscle (creatine)	Phosphorylation of creatine	Loss +
Leptin	Adipose tissue	Release from fat cell	Mass +

Loss = body condition loss; Mass = body mass; - = negative relationship; + = positive relationship.

2.5. Impact of pre-mating metabolic state on follicle development and piglet birth weight

As discussed above, sows' NEB during lactation have an impact on follicle size and development until ovulation and this follicle development has a major impact on piglet birth weight. Thus, pre-mating metabolic state, which is affected by sows' body condition losses during lactation, may also influence both follicle development and piglet birth weight.

During lactation, both LH secretion and follicle development are affected by sows' metabolic state. Lower feed intake during lactation result in less LH pulse during lactation (Quesnel et al., 1998a; van den Brand et al., 2000) than higher feed intake. In a study of Kauffold et al. (2008), sows in a restricted-fed diet during lactation had lower LH and follicle-stimulating hormone (FSH) concentrations during lactation and lower follicle diameters in late lactation. After weaning, a severe NEB during lactation resulted in a smaller follicle diameter (Quesnel et al., 1998a; Zak et al., 1997; van den Brand et al., 2000; Costermans et al., 2019b and 2020a) and lower ovarian weight (Quesnel et al., 1998a), which implies that not only follicle diameter but also the whole ovarian function is impaired. As discussed before, changes in LH pulse before and after weaning stimulate follicle development after weaning. However, when sows experience severe NEB, LH pulse is depressed also after weaning. For instance, sows with severe NEB had a low number of LH pulses before and after weaning and small follicle diameters (van den Brand et al., 2000). Insulin-like growth factor-1 is a major intermediate of energy balance that effects on follicle size and its development. At the hypothalamic level, plasma IGF-1 stimulates GnRH and thereby LH release. Van den Brand et al. (2001) found a positive relationship between IGF-1 and the number of LH pulses at and after weaning and follicle diameter at 2 days after weaning. Similarly, IGF-1 was positively related to basal LH concentration and LH surge level (Wientjes et al., 2012c). Insulin-like growth factor-1 acts also directly on the ovarian level by binding to the ovarian IGF-1 receptor (Liu et al., 2000). Follicle and oocyte development and subsequent embryo development are positively affected by the inclusion of IGF-1 during IVM (Xia et al., 1994). As the follicular fluid is part of the exudate of the blood (Gérard et al., 2002 and 2015), plasma IGF-1 largely impacts follicular fluid IGF-1. Costermans et al. (2020a) found a strong relation between plasma IGF-1 and follicular fluid IGF-1 concentration (β = 0.6 ng/ml per ng/ml $P \leq 0.0001$), indicating that plasma IGF-1 is an indicator for microenvironment of follicle and oocyte development. In this study (Costermans et al., 2020a), primiparous sows had smaller follicles at weaning after a severe feed restriction during

late lactation than full-fed sows. Also, they had smaller COCs in the follicles retrieved at 2 days after weaning which had a lower COCs expansion rate after 2 days of IVM and lower subsequent fertilization rate (Costermans et al., 2020a). Thus, pre-mating metabolic state has an impact on subsequent follicle development through changed IGF-1 concentrations, both at a systemic level and in the follicular fluid, and thereby on piglet birth weight.

Also other metabolic hormones and metabolites have been connected to follicle and oocyte development. In sows, creatinine concentration at weaning was negatively related to follicle diameters at weaning (Costermans et al., 2019b) and 2 days after (Costermans et al., 2020a), but this may be a direct association coming from a common cause, the NEB. In sows, NEFA (Quesnel et al., 1988b) and leptin concentrations (Costermans et al., 2020a) were associated with follicle diameter after weaning. In vitro studies have shown a direct influence of NEFA and leptin on follicle development. Higher NEFA in follicular fluid negatively affects oocyte developmental competence and blastocyst development after oocyte maturation in vitro in bovine (Leroy et al., 2005; van Hoeck et al., 2011). Barb et al. (2008) reviewed the role of leptin in the reproductive system of sows and concluded that leptin stimulates GnRH release in the hypothalamus, which can be beneficial for follicle development. Similarly, supplementation of leptin (10 and 100 ng/ml) has a positive impact on nuclear maturation in the porcine oocyte during IVM (Craig et al., 2005). Thus, similar to IGF-1, also these plasma metabolic hormones reflect their levels in follicular fluid, and affecting follicle and oocyte development, and possibly piglet birth weight.

In the past (before the 2000s), a severe NEB during lactation resulted in both prolonged WEI and poor subsequent fertility such as lower ovulation rate or embryo survival and subsequent lower litter size (Kemp et al., 2018). However, because of genetic selection for shorter WEI, severe NEB in modern sows hardly prolongs WEI, while ovulation rate and embryo survival may still be affected, especially in young sows with a generally higher NEB (Kemp et al., 2018). This implies that there may be different reproductive parameters with a similar WEI between sows with mild or severe NEB during lactation in hyper-prolific sows. Thus, metabolic hormones and metabolites before mating and during early pregnancy, seem to affect a cascade of processes that can impact foetal and placental development and thereby may be associated with piglet birth weight. Nevertheless, the extent to which specific lactation weight loss and related metabolic hormones and metabolites (IGF-1, NEFA, creatinine, urea and leptin) have an impact on subsequent litter characteristics has not been studied.

2.6. Impact of pre-mating diets on follicle development and piglet birth weight

As prevention of body condition loss during lactation is important for follicle and oocyte development, and embryo development, and even piglet birth weight in sows, optimizing sows' metabolic state during lactation is recommended. Several feeding strategies both during gestation and before ovulation have been studied for increasing piglet birth weight and decreasing within-litter piglet birth weight variation (reviewed by Campos et al., 2012; Wang et al., 2017; Peltoniemi et al., 2021b). Generally, the different feeding strategies during gestation have shown little impact on piglet birth weight and within-litter birth weight variation. Moreira et al. (2020) summarized 10 studies that investigated the effect of supplementation of amino acids (AA) during gestation on piglet birth weight and its variation. Supplementation of AA during gestation tended to reduce the SD in piglet birth weight of total born, but SD of piglet birth weight in born alive was not affected (Moreira et al., 2020). Also other litter characteristics, such as litter size, piglet birth weight and birth weight variation, were not affected by supplementation of AA during gestation. However, the pre-mating diet seems to have more beneficial impacts on subsequent piglet characteristics (i.e. litter size, birth weight and within-litter birth weight variation).

Table 2.2 shows the effects of various pre-mating diets on follicle development, oocyte quality, embryo development and litter characteristics at subsequent farrowing. Mainly the use of sugar sources has been studied as these are known to increase insulin and IGF-1 concentrations after feeding (Wientjes et al., 2012d). Van den Brand et al. (2006 and 2009) found that insulin-stimulating diets (inclusion of dextrose and lactose) before ovulation resulted in improved litter uniformity in sows (i.e. lower CV of piglet birth weight). However, the effects of insulin-stimulating diets on follicle development and subsequent fertilities have not been consistent (Table 2.2). This discrepancy may be due to different parity and pre-mating metabolic state of the sows.

Besides sugar-rich products, fibre sources (mainly sugar beet pulp (SBP)) have been used as pre-mating diets because fibres have been suggested to bind to E2 (Arts et al., 1991) in the gut, reducing circulating E2 (Ferguson et al., 2007), which stimulates gonadotropin release via negative feedback on the hypothalamic/pituitary axis (suggested by Ferguson et al., 2007). As a result of this, increased LH pulses were found in gilts fed SBP during the follicular phase in gilts (Ferguson et al., 2003). In addition, fibre-rich pre-mating diets resulted in higher proportions of oocytes in metaphase II *in vitro* and embryo survival at day 28 of pregnancy in gilts (Ferguson et al., 2007;

Weaver et al., 2013). Ferguson et al. (2004) also found that fibre-rich pre-mating diets increased litter size in multiparous sows. However, only a few studies have investigated the effects of fibre-rich diets in the pre-mating period on the subsequent litter characteristics and the mechanisms and effects remain largely unclear. The impact of SBP on follicle and oocyte development in sows is also inconsistent. Krogh et al. (2017) reported that supplementation of SBP (120 g/kg) in lactation diets decreased feed intake in the 3rd week of lactation compared with a commercial diet. The decreased feed intake may be connected to compromised follicle development and subsequent reproductive parameters. In addition to SBP, many different types of fibres with a range of physical and chemical properties are available (reviewed by Jarret and Ashworth, 2018). Microfibrillated cellulose (MFC) is processed through fibrillation of cellulose fibres and has been used as functional food ingredients for human nutrition (Serpa et al., 2016). Possibly, this MFC may act as a fibre-rich diet for sows, similar to high-fibre diets (Ferguson et al., 2007). Thus, the effects of different types of dietary fibre (DF) as pre-mating diets on sows' metabolic state and reproductive parameters remain still ambiguous and warrant investigations.

Based on the information provided in earlier chapters, the most relevant metabolic hormone for follicle development and subsequent fertility seems to be IGF-1. Increased IGF-1 concentrations can be achieved not only by dextrose and lactose but also by L-arginine (AR) and L-carnitine (LC). L-arginine is an essential AA for pigs and a precursor of biologically active molecules, such as polyamines and nitric oxide (Wu et al., 2010). Supplementation of AR increased IGF-1 concentration during lactation and gestation in sows (Zhu et al., 2017 – lactation; Guo et al., 2017 – gestation). In addition, supplementation of AR increased the percentage of monospermic fertilized oocytes (Hong and Lee, 2007), which is indicative of good oocyte quality, and blastocysts *in vitro* in pigs (Redel et al., 2015). L-carnitine is known to be involved in energy metabolism through transporting long-chain fatty acids into mitochondria. Numerous studies have shown that supplementation of LC increased IGF-1 concentration during gestation in sows (Musser et al., 1999; Birkenfeld et al., 2005; Doberenz et al., 2006). Also, supplementation of LC improved oocyte maturation and embryo development *in vitro* in pigs (Wu et al., 2011). Until now, the effects of AR and LC on follicle development have not yet been investigated.

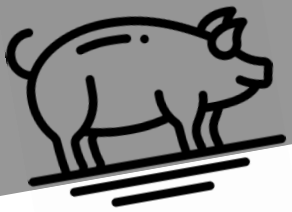
Table 2.2. Effects of pre-mating diets on the follicle and oocyte development, embryo development and litter characteristics in sows

Reference	Parity	Pre-mating diets	Feeding period	Follicle	Oocyte	Embryo	Litter characteristics				
							LS	PBW	CV	SD	PWM
Van den Brand et al. (2006)	P + M (3.0)	D	WEI				ns	ns	- *	ns	ns
Van den Brand et al. (2009)	M (5.4)	D + L	WL + WEI				ns	+ *	- *	- †	- *
Wientjes et al. (2012a)	M (4.6)	S + L	LD14+ WEI				ns	ns	ns	ns	
Wientjes et al. (2012b,c)	M (5.9)	D + L	From weaning until 12 h after ovulation	ns	ns	ns					
Wientjes et al. (2013b)	M (4.6)	D	WEI	ns		ns					
Chen et al. (2013)	P	D + S	LD9 + WEI				+ *	ns	ns	ns	
Quesnel et al. (2014)	M (3.0)	D (Arg during gestation)	WEI				ns	ns	ns	ns	
Chen et al. (2016)	P	D + S / Oil	LD7 + WEI				ns				
Plush et al. (2020)	M (3.1)	D	WEI				* +	ns	ns	ns	ns
Ferguson et al. (2003)	P + M (1 - 7)	Fibre (SBP)	LD14 + WEI				* +				
Ferguson et al. (2007)	G	Fibre (SBP)	21 days before insemination	+ Size * + FFv *	+ M2 *	+ Sr *					
Weaver et al. (2012)	G	Fibre (Lupin)	3 weeks before puberty until days 19 of 1st oestrus		+ M2 *	+ Sr *					
Gianluppi et al. (2020)	P+ M (3.0)	Lactation diet[1]	WEI				ns	ns			

PBW = mean piglet birth weight; CV = CV birth weight; SD = SD birth weight; G = gilts; P = primiparous sows; M = multiparous sows; D = dextrose; L = lactose; S = sucrose; Arg = arginine; ns = not significant; WL = whole lactation period; LD14 = 14 days before weaning; LD9 = 9 days before weaning; LD7 = 7 days before weaning; ns = not significant; + = increased; - = decreased; * = significant ($P < 0.05$); † = tendency ($P < 0.10$); SBP = sugar beet pulp; FFv = follicular fluid volume; M2 = metaphase 2; Sr = survival rate; LS = litter size; PWM = pre-weaning mortality.

[1]Replace gestation diet with lactation diet.

3



3. AIMS AND HYPOTHESES OF THE STUDY

The aim of this thesis was to evaluate the effects of different types of pre-mating diets on young sows' metabolic state, follicle development and litter characteristics at subsequent farrowing. In addition, we investigated how profiles of metabolic hormones and metabolites during the WEI are related to body condition loss during lactation and to subsequent follicle development and litter characteristics.

We hypothesized that supplementation of amino acids ($_{L}$-arginine and $_{L}$-carnitine) in pre-mating diets improves IGF-1 and follicle development, and dietary fibre (microfibrillated cellulose and sugar beet pulp) supplemented to pre-mating diets improves follicle development before ovulation, and thereby both can positively affect piglet birth weight at subsequent farrowing. Furthermore, we hypothesized that the sows' lower body condition loss during lactation positively affects pre-mating metabolic state, which carries over a positive effect to follicular development before ovulation and to subsequent litter characteristics.

Specific aims and hypotheses are as following:

1. We aim to evaluate the effects of different types of pre-mating diets during the last week of lactation on metabolic state after weaning in young sows. We hypothesize that dietary supplementations of L-arginine (AR) and L-carnitine (LC) increase IGF-1 concentration and microfibrillated cellulose (MFC) and sugar beet pulp (SBP) affect feed intake during lactation and thereby metabolic hormones (IGF-1, NEFA, creatinine and leptin) during the WEI.

2. We aim to evaluate the effects of different types of pre-mating diets during the last week of lactation on follicle development after weaning in young sows. We hypothesize that dietary supplementations of AR, LC, MFC and SBP increase follicle diameters during the WEI.

3. We aim to evaluate the effects of different types of pre-mating diets during the last week of lactation on follicle development after weaning in young sows. We hypothesize that dietary supplementations of AR, LC, MFC and SBP increase piglet mean birth weight and decrease the proportions of small piglets via a carry-over effect of pre-mating diets on follicle development.

4. We aim to investigate how profiles of metabolic hormones and metabolites during the WEI affect follicle development in young sows. We hypothesize that concentrations of metabolic hormones and metabolites (IGF-1, NEFA, creatinine and leptin) related to higher body condition losses during lactation has a negative correlation with follicle diameters and subsequent reproductive parameters.

5. We aim to investigate how profiles of metabolic hormones and metabolites during the WEI affect piglet birth weight at subsequent farrowing in young sows. We hypothesize that concentrations of metabolic hormones and metabolites (IGF-1, NEFA, creatinine and leptin) related to higher body condition losses during lactation have a negative correlation with piglet birth weight and litter uniformity.

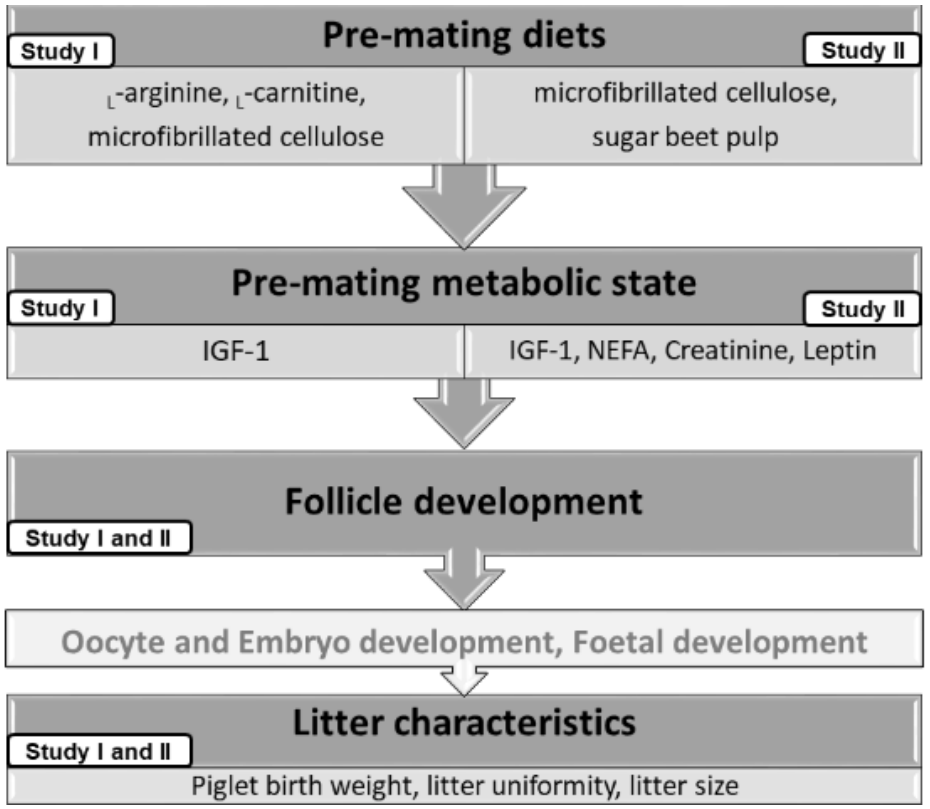
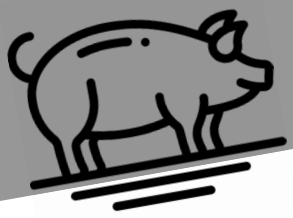


Fig. 3.1. Outline of the PhD thesis. Study I aimed to investigate the effects of L-arginine (AR), L-carnitine (LC) and microfibrillated cellulose (MFC), and Study II the effects of dietary fibre (MFC and sugar beet pulp (SBP)) on metabolic states, follicle development and litter characteristics. Further, this thesis investigated if and how pre-mating metabolic state is related to follicle development after weaning and piglet birth weight at subsequent farrowing.

4



4. MATERIAL AND METHODS

4.1. Animals, housing and management

An overview of animals, breeds, parity and dietary treatment in both studies is presented in Table 4.1. Study I and II were conducted on a research herd in western Finland. In Study I, first-parity (N = 56) and second-parity (N = 20) sows were used in three consecutive batches (N = 23, N = 30 and N = 23, respectively). Of these sows, 41 first-parity and 15 second-parity sows showed oestrus and farrowed subsequently (oestrus rate was on average 81.6%; N = 20, N = 20 and N = 16, respectively). In Study II, first-parity sows (N = 58) were used in three consecutive batches (N = 21, N = 22 and N = 15, respectively). Of these sows, 44 first-parity sows showed oestrus and farrowed subsequently (oestrus rate was on average 86.0%; N = 14, N = 18 and N = 12, respectively).

Table 4.1. Overview of the animals used in Study I and II

Study	Paper	Parity	No. of sows	Breeds	Dietary treatments
I	I	1	56	DanAvl (YLY or LYL)	Control (CON), L-arginine (AR), L-carnitine (LC), Microfibrillated cellulose (MFC)
		2	20		
	II	1	41		
		2	15		
II	III	1	58		Control (CON), Microfibrillated cellulose (MFC), Sugar beet pulp (SBP)
	IV		44		

Housing and management were similar between studies, unless otherwise stated. One week prior to parturition, sows were transferred to the farrowing and lactation unit where they were housed in individual farrowing crates. Within 2 days after farrowing, litters were standardized based on the number of functional teats. After weaning at day 26.1 ± 0.2 (Study I) and day 29.7 ± 0.4 (Study II) of lactation, the sows were moved to the insemination units with individual stalls. From weaning onwards, oestrus detection was performed once daily at 1200 h (Study I) or twice daily at 1100 and 1800 h (Study II) by a farm technician using fence-line boar contact.

In both studies, sows were artificially inseminated once on every day of oestrus with a commercial dose of semen (mostly for two consecutive days; 2×10^9 sperm cells; DanAvl; Finnpig, Finland). Pregnancy check with ultrasound was performed by a farm technician 35 days after the first insemination. Sows were then moved to the gestation unit with a group housing system (4 to 6 sows per pen). One week prior to subsequent parturition, sows were transferred to the farrowing and lactation unit.

4.2. Lactation and gestation feeding

In both studies, sows were provided with liquid feed (1:3.35, feed to water ratio) and water ad libitum in the farrowing and lactation unit. Feed composition and feed allowance during lactation and gestation differed between studies due to changes in feeding curves. Before farrowing and in the first 2 weeks of lactation, sows received a standard commercial lactation diet (9.2 MJ net energy (NE)/kg DM, 13.8% CP, Imetys Pekoni 1; Hankkija Oy, Hyvinkää, Finland) twice a day (0730 and 1300 h). After 2 weeks of lactation, sows received another lactation diet until weaning (9.9 MJ NE/kg DM, 15.3 (Study I) or 16.0% (Study II) CP, Imetys Pekoni 2; Hankkija Oy, Hyvinkää, Finland) four times a day (0330, 0730, 1300 and 1800 h).

The dry feed allowance before farrowing was 2.99 kg/day and gradually increased to 7.45 (Study I) or 6.89 kg/day (Study II) during the first 2 weeks of lactation. After the first 2 weeks of lactation, the maximum feed allowance was 7.45 (Study I) or 7.21 kg/day (Study II). From weaning until oestrus, sows were fed 4.6 (Study I) or 4.82 kg/day (Study II) of a commercial gestation diet 1 (9.0 (Study I) or 9.1 (Study II) MJ NE/kg DM, 11.5% (Study I) or 12.3 % (Study II) CP, Tiineys Pekoni 1; Hankkija Oy, Hyvinkää, Finland) twice a day (0700 and 2000 h).

From day 0 of gestation (day of the first insemination) to day 35 of gestation, sows were fed 3.37 (Study I) or 3.30 kg/day (Study II) of commercial gestation diet 1. From day 35 to day 40 of gestation, sows were fed 3.26 (Study) or 3.19 kg/day (Study II) of commercial gestation diet 1. From day 40 to day 84 of gestation, sows were fed 2.63 (Study I) or 2.45 kg/day (Study II) of commercial gestation diet 2 (9.1 MJ NE/kg DM, 13.0% CP for both studies, Tiineys Pekoni 2; Hankkija Oy, Hyvinkää, Finland). From day 84 of gestation until transfer to the farrowing unit, sows were fed 3.68 kg/day of commercial gestation diet 2 (both studies).

4.3. Dietary treatment

Sows received one of the seven (Study I) or three (Study II) dietary treatments during the last week of lactation and the WEI. Allocation to treatments was stratified based on parity, BW loss (kg) between 1 day after farrowing and treatment allocation and number of piglets at allocation.

In Study I, sows received once daily a top dressing of 200 g, consisting of either wheat (CON) or wheat plus MFC (Betulium® Microfibrillated cellulose, Espoo, Finland), L-carnitine (LC; CarnikingTM, Lonza Group, Inc., Allendale, NJ, USA) or L-arginine (AR; L-arginine, Cheiljedang, Indonesia) at one of two supplementation levels during the last week of lactation (Table 4.2) and WEI (Table 4.3). In detail, AR supplementation level was comprised to 1% or 1.5% of the total feed amount, which is similar or higher than Zhu et al. (2017), who found higher IGF-1 on the 3rd week of lactation in sows fed 1.0% AR (Tables 4.2 and 4.3). The supplementation level of LC was similar or higher than Musser et al. (1999) who found increased IGF-1 concentration at days 60 and 90 of pregnancy in sows fed 0.23 kg/day of LC (Tables 4.2 and 4.3). The supplementation level of MFC was designed to be similar to the pilot trial with broilers in the feed company (0.1% or 0.2% of total feed intake; Tables 4.2 and 4.3).

Table 4.2. Composition of the experimental top-dressed diet of sows during 1 week before weaning in Study I

Ingredients	CON	MFC1	MFC2	LC1	LC2	AR1	AR2
Wheat, g	200.00	192.50	185.00	199.75	199.62	125.50	88.20
Microfibrillated cellulose, g	-	7.50	15.00	-	-	-	-
L-carnitine, g	-	-	-	0.25	0.38	-	-
L-arginine, g	-	-	-	-	-	74.50	111.80
Total, g	200.00	200.00	200.00	200.00	200.00	200.00	200.00
Analysed value, %							
Dry matter	89.9	89.5	89.7	89.1	89.2	92.7	94.4
Crude protein	16.8	16.2	15.6	16.8	16.9	77.4	93.9
Crude fat	5.4	5.5	5.4	5.7	5.6	3.3	2.7
Crude fibre	7.7	9.2	11.6	7.7	7.5	5.3	3.8
Crude ash	4.4	4.5	4.7	4.3	4.4	2.8	2.1

Table 4.3. Composition of the experimental top-dressed diet of sows during the weaning-to-oestrus interval in Study I

Ingredients	CON	MFC1	MFC2	LC1	LC2	AR1	AR2
Wheat, g	200.00	195.40	190.80	199.77	199.65	153.80	130.70
Microfibrillated cellulose, g	-	4.60	9.20	-	-	-	-
L-carnitine, g	-	-	-	0.23	0.35	-	-
L-arginine, g	-	-	-	-	-	46.20	69.30
Total, g	200.00	200.00	200.00	200.0	200.0	200.00	200.00
Analysed value, %							
Dry matter	89.9	89.5	89.7	89.4	89.3	91.6	92.6
Crude protein	16.8	16.0	16.0	16.2	16.2	56.9	75.5
Crude fat	5.4	5.1	5.1	5.3	5.2	4.0	3.3
Crude fibre	7.7	8.9	10.4	7.6	8.5	6.5	5.0
Crude ash	4.4	4.4	4.4	4.2	4.5	3.4	2.8

In Study II, control group (CON; commercial diet) and MFC sows received 7.73 kg/day of dry feed allowance during 1 week before weaning. Fifty grams of commercial diets were replaced with 50 g of MFC (Betulium® Microfibrillated cellulose, Espoo, Finland; Table 1) for MFC sows. The supplementation level of MFC in Study II was intended to be at a higher level than in Study I (4.6-15 g) because there was no effect of MFC on sows feed intake and reproductive parameters in Study I. Sugar beet pulp sows were fed 7.81 kg/day of dry feed allowance in which 520 g of commercial diets were replaced with 600 g of SBP-rich diet (Table 4.4), without affecting net energy (NE) intake. The supplementation level of SBP in Study II was lower than Krogh et al. (2017), who found that 120 g/kg of SBP in lactation diets had no detrimental impact on feed intake during late lactation in multiparous sows. During lactation, daily feed allowance of individual sows was reduced by 10% when feed residuals remained in the trough. During the WEI, CON sows were provided 4.79 kg/day of commercial gestation diet (Tiineys Pekoni 1; Hankkija Oy, Hyvinkää, Finland). The MFC sows were provided 4.79 kg/day in which 50 g of commercial gestation diet was replaced with 50 g of MFC (Table 1) and SBP sows were provided 4.82 kg/day in which 570 g of commercial gestation diet was replaced with 600 g of SBP-rich diet (Table 4.4), without affecting NE intake.

The experimental diets were analysed for DM (EU 152/2009), CP (Dumas methods), crude fat (EU 98/64), crude fibre (EU 92/89), NDF (AOAC 2002:04/ISO 16472:2005), ADF (ISO 13906:2008), non-starch polysaccharides (NSP; similar to Jonathan et al., 2013), lignin (ANKOM method), and Ca and P (ISO 11885:2009).

Table 4.4. Chemical composition of the commercial diets (CON), sugar beet pulp (SBP)-rich diet and microfibrillated cellulose (MFC) of sows in Study II

Ingredients	CON lactation[1]	CON gestation[1]	SBP[1]	MFC[1]
Analysed composition, g/kg, as-is basis				
DM, %	87.8	87.1	89.6	97.2
CP, g/kg	168	129	127	89
Crude fat, g/kg	51	32	42	3
Crude fibre, g/kg	51	73	152	199
NSP, g/kg	108	152	412	360
ADL, g/kg	17	17	23	65
Total dietary fibre, g/kg[2]	125	179	435	425
NDF, g/kg	155	208	290	400
ADF, g/kg	42	86	159	250
Ca, g/kg	9.1	6.6	16.0	7.3
P, g/kg	5.5	3.5	3.3	0.7

NE = Net energy; NSP = Non-starch polysaccharides; ADL = Acid detergent lignin; NDF = Neutral detergent fibre; ADF = Acid detergent fibre.

[1]600g of SBP replaced 520 g of CON lactation diet and 570 g of CON gestation diet, respectively. 50 g of MFC replaced 50 g of both CON lactation and gestation diets.

[2]Sum of non-starch polysaccharides and ADL.

4.3. Body weight, backfat and loin muscle depth

Sows' BW, BF and LM were measured 1 day after farrowing, 1 week before weaning and at weaning (both studies; Fig. 4.1). Backfat thickness and LM were measured at P2 on the right and left side of the sow (at 6 cm from the midline straight above the last rib bone) using a B-mode ultrasound with a 10.0 MHz linear array probe (MyLab One VET; Esaote, The Netherlands). Backfat thickness was measured as the length from the skin to muscle layer and LM was measured as the length between the fat layer and rib bone (Fig. 4.1). Backfat thickness and LM were measured at two different points within each ultrasound image and averaged.

At subsequent farrowing, sows' BW was calculated as BW at entering the farrowing unit deducted by the sum of the estimated weight of the foetuses, placentas and intrauterine fluid, using the equations of Bergsma et al. (2009).

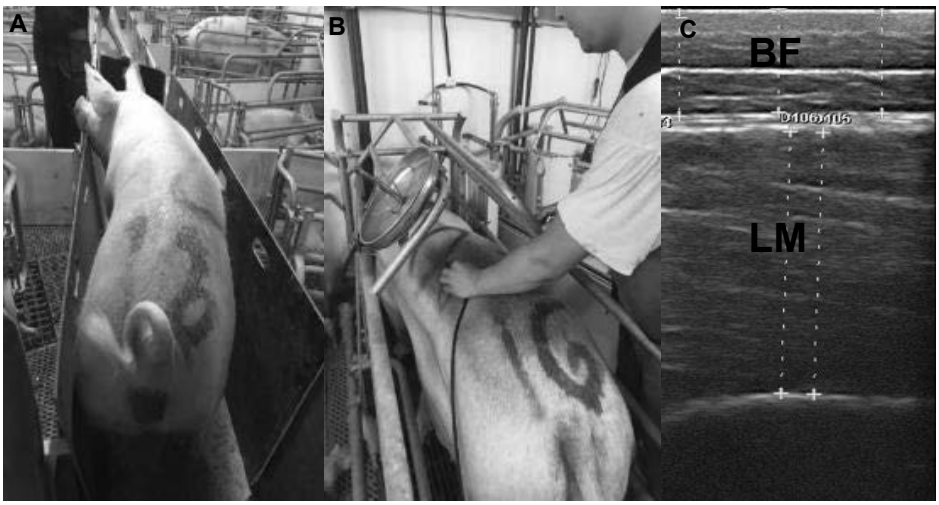


Fig. 4.1. Measurement of body weight (A), measurement of backfat thickness (BF) and loin muscle depth (LM) at P2 on the right and left side of the sow (B) and determination of BF and LM in the ultrasound image (C).

4.4. Blood sampling and analysis

Blood samples for IGF-1 (both studies) were taken from the vena coccygea at 1 week before weaning, at weaning, at 3 days after weaning and on the second day of oestrus (Fig. 4.2). In Study II, blood samples for NEFA, creatinine, urea and leptin were taken from the vena coccygea before feeding at 1 week before weaning, at weaning, 3 days after weaning and second day of oestrus (Fig. 4.2). Blood samples for E2 (Study II) were collected on the day of weaning, at 3 days after weaning and on the second day of oestrus (Fig. 4.2).

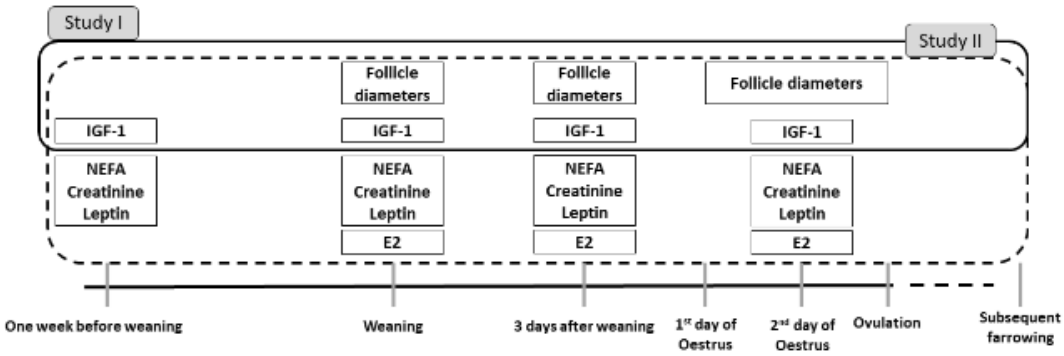


Fig. 4.2. Schematic illustration of sampling period of blood and ovarian ultrasonography in Study I (solid line) and II (dashed line).

The samples for IGF-1 were collected in 3-ml EDTA tubes (VACUETTE® K2EDT, Greiner Bio-One Italia, Cassina de Pecchi, Italy), immediately placed on ice and centrifuged at 1710×g for 10 min at 4°C. Plasma was stored at −20°C until analyses. In Study II, blood samples were collected into 3-ml serum tubes (VACUETTE® K2EDT, Greiner Bio-One Italia, Cassina de Pecchi, Italy).

Sensitivity and intra- and inter-assay coefficients of variation for IGF-1, NEFA, creatinine, urea, leptin and E2 were within a range indicating good functionality of the assays performed and they are presented in the original articles.

IGF-1 concentrations were analysed using a commercial kit (IRMA IGF-1 A15729®; Immunotech, Marseille, France) after extraction of the samples with ethanol and HCl (as validated by Louveau and Bonneau, 1996). A commercial kit was used (Randox NEFA kit; Randox Laboratories Ltd.,

Crumlin, UK) for the NEFA analysis. Creatinine and urea were analysed using the same a commercial kit (Konelab™ / T Series CREATININE (Enzymatic) 981896, Thermo Fisher Scientific, Vantaa, Finland). Leptin concentration was analysed using a commercial kit (Multi-Species Leptin XL-85 K, EMD Millipore Corporation, Billerica, MA, USA). Serum E2 concentrations were analysed by using an Ultra-sensitive Estradiol RIA (Beckman Coulter, Brea, CA, USA) with ether extraction. In detail, 1 ml of plasma sample was extracted with 5 ml of diethyl ether (AnalR Normapur PDH, Prolabo, Leuven, Belgium). Extraction was repeated twice. Dried extracts were re-suspended into 200 µl of 0.1% gelatine in PBS, and E2 was measured by using the commercial kit mentioned above.

4.5. Follicle development, oestrus and ovulation

Trans-rectal ultrasonography (Fig. 4.3) with an 8-MHz linear array probe (MyLab One VET; Esaote, The Netherlands) was performed to assess follicle diameter on the day of weaning, at 3 days after weaning and at 12-h intervals during oestrus until ovulation (Fig. 4.2). Ultrasound clips were taken from only one ovary because their ovarian function represents bilateral finding in the ovary in sows (Soede N. M., unpublished results). The clips (Fig. 4.4) were exported in DICOM format and analysed using the DICOM viewer Horos (version 3.3.2, available at www.horosproject.org). Follicle diameter was defined as the mean diameter of the five largest follicles. The largest measured follicle diameter during oestrus was defined as the follicle diameter at ovulation.

The time interval between weaning and first onset of oestrus (standing response) was regarded as the weaning-to-oestrus interval (WEI). The time of ovulation was defined as 6 h before the first scan when no pre-ovulatory follicles were found to calculate the weaning-to-ovulation interval (WOI). The oestrus rate was calculated as the percentage of sows showing oestrus within 7 days after weaning.

Fig. 4.3. Trans-rectal ultrasonography for assessing follicle diameters.

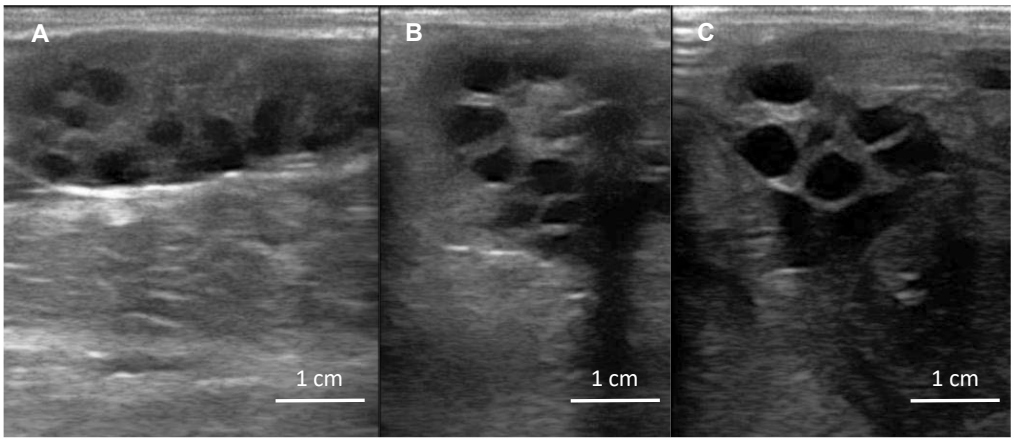


Fig. 4.4. Ultrasound image of illustrating growth of follicles at weaning (A), at 3 days after weaning (B) and at oestrus (C).

4.6. Litter characteristics

The number of live born and stillborn piglets were assessed at subsequent farrowing. The total number of piglets born per litters was calculated by summing the number of live born and stillborn piglets. All live born and stillborn piglets were weighed individually within 12 h after birth and standard deviation (SD) and coefficient of variation (CV) of birth weight were calculated for these piglets (Fig. 4.5). The proportions of piglets weighing < 1 000 g and > 1 800 g in the same litters were calculated. Sows with ≥ 8 piglets were included in the analysis.

Fig. 4.5. Measurement of individual piglet birth weight.

4.7. Statistical analysis

SAS 9.4 (SAS Institute. Inc., Cary, NC, USA) was used for statistical analyses of all data. Normality of the parameters was checked with the UNIVARIATE procedure using the Shapiro–Wilk test. In both studies, the normally distributed parameters (IGF-1 concentrations, follicle diameter, total number born, litter weight at birth, mean birth weight, SD and CV of birth weight) were analysed with the MIXED procedure (model 1). Non-normally distributed parameters (WEI, WOI, oestrus duration, oestrus and pregnancy rate, NEFA, creatinine, urea, leptin, E2 concentrations, proportions of piglets < 1 000 g and piglets > 1 800 g) were analysed with the GLIMMIX procedure (model 2). In Study I, treatment (CON, MF1, MF2, LC1, LC2, AR1, AR2), parity (1, 2), and their interaction were used as fixed effect. In Study II, treatment (CON, SBP, MFC) was used as fixed effect. In both studies, preliminary analyses showed that batches (1, 2, 3) and breeds (YLY, LYL) were never significant and thus were used as a random effect to account for possible environmental and genetic variation in both models 1 and 2. Tukey–Kramer corrections were used for multiple comparisons. Significance was regarded as $P \leq 0.05$. Normally distributed parameters were presented as least-square means, and non-normally distributed parameters were presented as means.

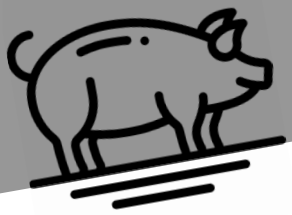
In Study I, repeated measure was used in model 1 to assess the effects of body condition changes during lactation classes (high LM = loin muscle depth loss during lactation ≥8%, N = 39; low LM= loin muscle depth loss during lactation <8%, N = 37), parity and their interaction on IGF-1 concentrations at weaning, 3 days after weaning and oestrus to assess the effect of LM loss on IGF-1 concentrations. Insulin-like growth factor-1 concentrations at the start of the treatment were used as a covariate.

A gamma distribution with a log link function was fitted to model 2 for WEI, WOI, oestrus-to-ovulation interval (EOI), NEFA, creatinine, leptin and E2 concentrations. For oestrus and pregnancy rate and proportions of piglets < 1 000 g and > 1 800 g, a binomial distribution with a logit link function was fitted to model 2 for both studies.

Pearson and Spearman correlations were used for assessing correlations among normally distributed and non-normally distributed parameters, respectively. In Study I, relations between parameters were estimated using the following model: $Y_{ij} = \mu + PAR + \beta X_{ij} + \beta X \times PAR + \epsilon_{ij}$, where Y_{ij}

is the dependent variable, PAR is the parity (1, 2), β is the regression coefficient and X_{ij} is the independent variable. The interactions were excluded from models when not significant. In Study II, relationships between parameters were estimated using the following model: $Y = \mu + \beta X + \varepsilon$, where Y is the dependent variable, β is the regression coefficient and X is the independent variable. In both models, treatment, batch and breed were included as random effects to account for possible treatment effect, and environmental and genetic variation.

5



5. RESULTS

5.1. Effect of pre-mating diets on metabolic state

Average daily feed intake and body condition losses (i.e. BW, BF and LM) from starting treatment to weaning (i.e. 1 week before weaning) did not differ between treatment (Study I and II) and parity (Study I; Table 5.1). However, SBP sows had higher BF loss than MFC sows during 1 week before weaning, whereas they were similar to CON sows (0.6 v. 1.1 v. 0.2 mm, for CON, SBP and MFC, respectively, $P < 0.05$).

Table 5.1. Descriptive data of lactation length, average daily feed intake (ADFI) and body condition losses during lactation in sows in Study I and II (mean ± SE)

	Study I		Study II
	Parity 1	Parity 2	Parity 1
Number of sows, n	56	20	58
Lactation length, day	26.2 ± 0.2	25.8 ± 0.3	29.7 ± 0.4
ADFI during lactation, kg/day	4.7 ± 0.1	4.9 ± 0.1	5.3 ± 0.1
Body weight			
Loss during lactation, kg	26 ± 2	30 ± 5	17 ± 2
Loss during lactation, %	12.2 ± 1.0	11.8 ± 1.8	7.8 ± 0.8
Backfat			
Loss during lactation, mm	3.3 ± 0.2	2.8 ± 0.4	3.2 ± 0.2
Loss during lactation, %	22.8 ± 1.8	18.5 ± 2.3	23.5 ± 1.5
Loin muscle depth			
Loss during lactation, mm	4.8 ± 0.7	1.9 ± 1.5	6.2 ± 0.7
Loss during lactation, %	9.0 ± 1.3	2.9 ± 2.9	11.7 ± 1.2

Insulin-like growth factor-1 concentrations between weaning and oestrus, that were corrected for pre-treatment IGF-1 concentrations, were not affected by treatment (Study I and II; Fig. 5.1A and B) or by parity (Study I). In Study I, IGF-1 concentrations were lowest at weaning, thereafter increasing (199 v. 265 v. 265 ng/ml, $P < 0.001$; Fig. 5.1A). In Study II, however, IGF-1 concentrations were similar throughout WEI (231 v. 249 v. 225 ng/ml for at weaning, at 3 days after weaning and at oestrus, respectively, $P > 0.05$; Fig. 5.1B). In both studies, IGF-1 concentrations at 1 week before weaning, at weaning, at 3 days after weaning and at oestrus were positively correlated to each other ($r \geq 0.38$, $P \leq 0.001$ for all).

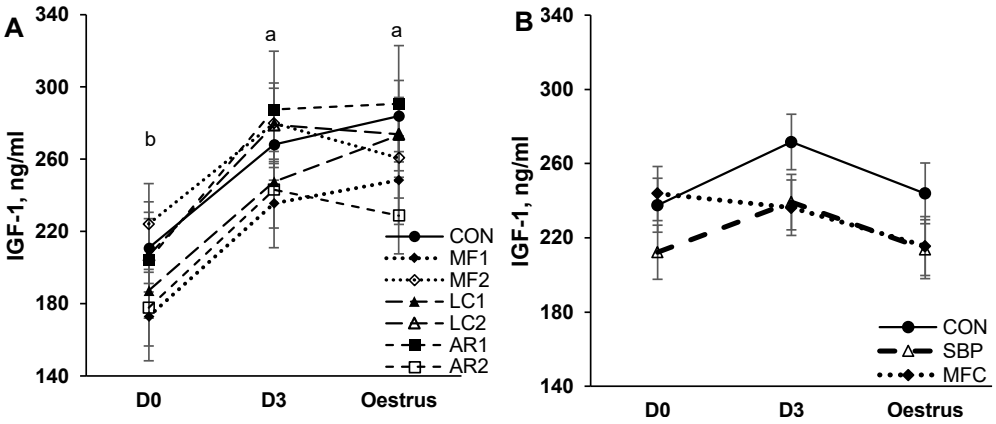


Fig. 5.1. Plasma IGF-1 (ng/ml) profiles at weaning (D0), 3 days after weaning (D3) and at oestrus corrected for pre-treatment concentrations in sows fed pre-mating diets during 1 week before weaning and the weaning-to-oestrus interval in Study I (A) and Study II (B). No differences in IGF-1 concentrations were found between parities in Study I ($P > 0.05$). [a,b,c] days with different superscript differ, $P \leq 0.001$.

Serum NEFA and leptin concentrations were not affected by treatment in Study II (Fig. 5.2 A and C). Creatinine concentration at weaning was higher in SBP sows than in MFC sows (162.9 v. 136.6 mmol/L, P < 0.001; Fig. 5.2B). After weaning, creatinine concentrations were not affected by treatment (Fig. 5.2B). Both NEFA and creatinine concentrations were highest at weaning, decreasing thereafter (P < 0.001; Fig. 5.2A, B). Leptin concentrations were lowest at weaning, increasing thereafter (P < 0.001; Fig. 5.2C).

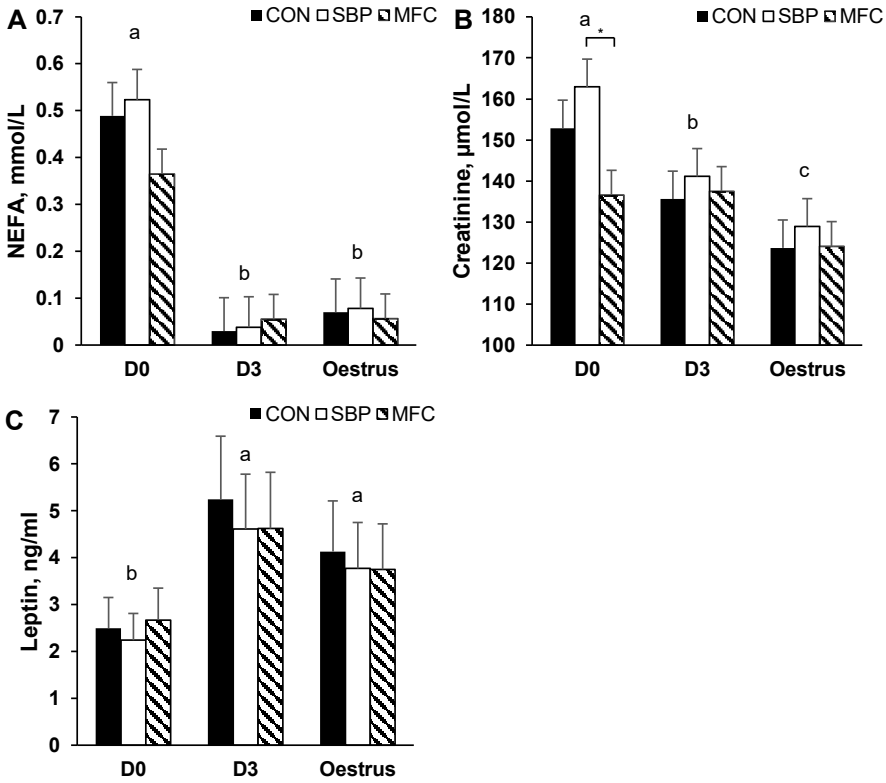


Fig. 5.2. Serum NEFA (A; mmol/L), creatinine (B; µmol/L) and leptin (C; ng/ml) profiles at weaning (D0), 3 days after weaning (D3) and at oestrus corrected for pre-treatment concentrations in sows fed either commercial diet (CON), sugar beet pulp (SBP)-rich diet or microfibrillated cellulose (MFC) during 1 week before weaning and the weaning-to-oestrus interval (Study II). *P ≤ 0.001, ^{a,b,c} days with different superscript differ, P ≤ 0.001.

5.2. Effect of pre-mating diets on follicle development

First-parity sows tended to have a smaller follicle diameter during the WOI than second-parity sows in Study I ($P \leq 0.10$ for all). However, WOI did not differ between parities (Table 5.2). Follicle diameters during the WOI, oestrus and ovulation characteristics and pregnancy rate at 35 days after the first insemination were not affected by treatment in the two studies. Similar E2 concentrations were observed between treatments in Study II.

Table 5.2. Descriptive data of follicle development and oestrus and ovulation characteristics in sows in Study I and II (mean ± SE)

	Study I		Study II
	Parity 1	Parity 2	Parity 1
Number of sows, n	56	20	58
Follicle diameters, mm[1]			
At weaning	3.6 ± 0.1	3.8 ± 0.1	4.0 ± 0.1
At 3 day after weaning	6.0 ± 0.1	6.4 ± 0.2	6.5 ± 0.1
At ovulation	6.9 ± 0.1	7.2 ± 0.1	7.3 ± 0.1
Oestrus rate ≤ 7 days, %	80.4 ± 5.4	90.0 ± 6.9	82.8 ± 5.0
WOI, h	140.5 ± 2.4	136.0 ± 4.6	129.1 ± 1.5

[1] The 5 largest follicles in one ovary. Follicle diameters at ovulation was determined as the largest follicles during oestrus.

5.3. Effect of pre-mating diets on litter characteristics

Descriptive data of litter characteristics in both studies are presented in Table 5.3. Pre-mating diets did not affect litter characteristics in neither Study. In Study I, first-parity sows had a lower total number of piglets born per litter ($P = 0.02$), higher mean piglet birth weight ($P < 0.01$), lower CV of piglet birth weight ($P < 0.01$), and lower proportions of piglets < 1 000 g ($P < 0.01$) at subsequent farrowing than second-parity sows. This was still the case when piglet birth weight was corrected for litter size.

Table 5.3. Descriptive data of litter characteristics in sows in Study I and II (mean ± SE)

	Study I		Study II
	Parity 1	Parity 2	Parity 1
Number of sows, n	41	15	44
Litter characteristics			
Total born, n	18.5 ± 0.5	20.3 ± 0.8	19.6 ± 0.6
Litter weight at birth, kg	25.2 ± 0.6	24.1 ± 0.8	25.6 ± 0.7
Mean birth weight, g	1384 ± 31	1205 ± 49	1344 ± 33
SD of birth weight, g	276 ± 10.3	310 ± 12.0	270 ± 0.8
CV of birth weight, %	20.2 ± 0.8	26.3 ± 1.4	20.7 ± 0.8
Piglets < 1 000 g, %	13.1 ± 1.9	27.0 ± 4.0	16.3 ± 1.9
Piglets > 1 800 g, %	10.1 ± 2.5	4.2 ± 2.2	7.7 ± 2.6

5.4. Relationships between metabolic state and follicle development

IGF-1 at weaning was negatively related to the percentage of BW loss, BF loss and LM loss (β ≤ −3, −2 and −2 ng/ml per %, respectively; P < 0.05 for all) in both studies. However, no relationships were found between body condition loss and IGF-1 levels at 3 days after weaning or at oestrus. In Study I, first-parity sows with high LM loss had lower IGF-1 concentrations at weaning (167 ± 13 ng/ml) than first-parity sows with low LM loss (214 ± 13 ng/ml), and second-parity sows with high (225 ± 23 ng/ml) and low LM loss (221 ± 16 ng/ml; P < 0.05; Fig. 5.3).

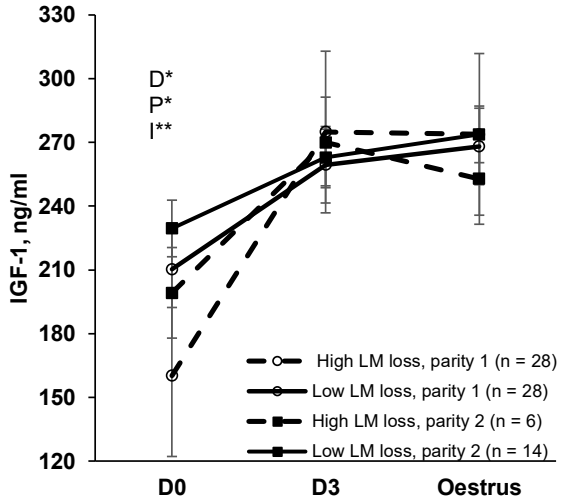


Fig. 5.3. Effects of parity, and high and low loin muscle depth (LM) loss during lactation on treatment corrected plasma IGF-1 concentrations during the weaning-to-oestrus interval (WEI) of sows in Study I. D0 = at weaning; D3 = at 3 days after weaning, High LM loss = loin muscle depth loss during lactation ≥ 8%; Low LM loss = loin muscle depth loss during lactation < 8%; Day effect (D); D0 differs from D3 and oestrus; P ≤ 0.05; Parity effect (P); P ≤ 0.05, Interaction (I) between parity and loin muscle depth loss during lactation; P ≤ 0.01.

In Study I, IGF-1 concentrations varied from 60 to 311 ng/ml at weaning. Within parity, an increase in IGF-1 concentration was accompanied by a 0.5 mm difference in follicle diameter at weaning (β = 0.002 mm per ng/ml, P < 0.0001; Fig. 5.4A) and at 3 days after weaning (β = 0.002 mm per ng/ml, P = 0.06). Also in Study II, higher plasma IGF-1 concentration at weaning was related to larger follicle

diameters at weaning (β = 0.001 mm per ng/ml, P < 0.01; Fig. 5.4B). Higher IGF-1 concentrations at weaning and 3 days after weaning were related to shorter WOI in both studies (β ≤ -0.03 h per ng/ml, P < 0.05 for all).

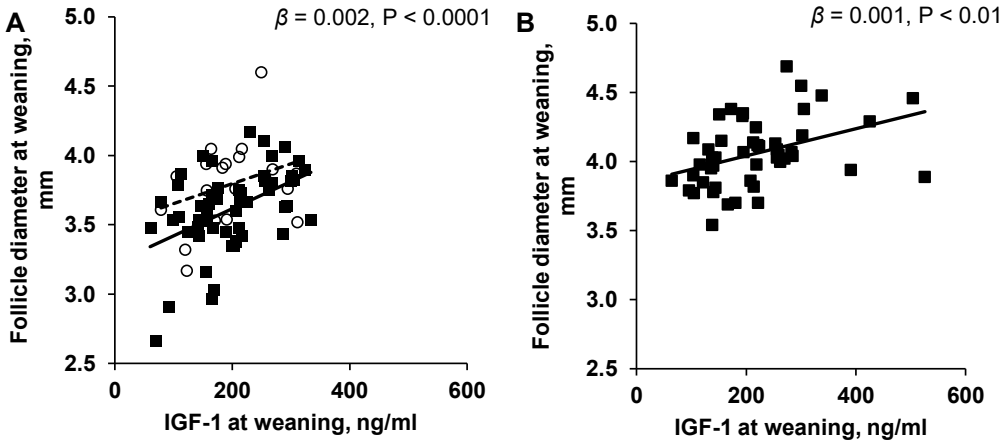


Fig. 5.4. Regression equations (β) for the relations between IGF-1 concentrations at weaning and follicle diameter of the five largest follicles at weaning in first- (■, solid line) and second-parity sows (○, dotted line) in Study I (A) and first-parity sows in Study II (B). No interactions with parity were observed in Study I.

At 3 days after weaning, leptin concentration and follicle diameters were positively related to each other in Study II (β = 0.08 mm per ng/ml, P < 0.05; Fig. 5.5B).

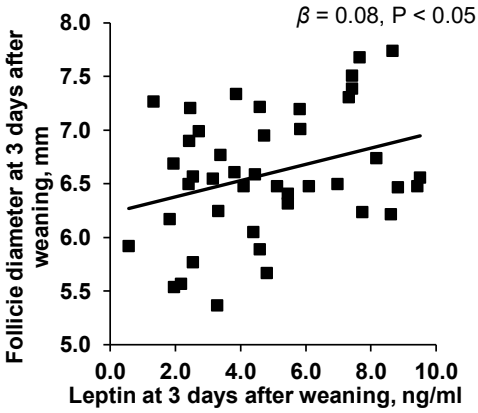


Fig. 5.5. Regression equations (β) for the relations between leptin concentration and mean follicle diameters at 3 days after weaning in first-parity sows in Study II.

5.5. Relationships between metabolic state and litter characteristics

IGF-1 concentration during WEI was not related to the total number of piglets born per litter in either study. However, in first-parity sows, IGF-1 concentration at weaning was positively related to litter weight at birth at subsequent farrowing in both studies (β = 0.02 kg per ng/ml, P < 0.01

and β = 0.01 kg per ng/ml, P < 0.06, respectively for Study I and II; Fig. 5.6A and B). In Study II, this relation was also seen for IGF-1 concentrations at 3 days after weaning and at oestrus (β ≥ 0.02 kg per ng/ml, P ≤ 0.02).

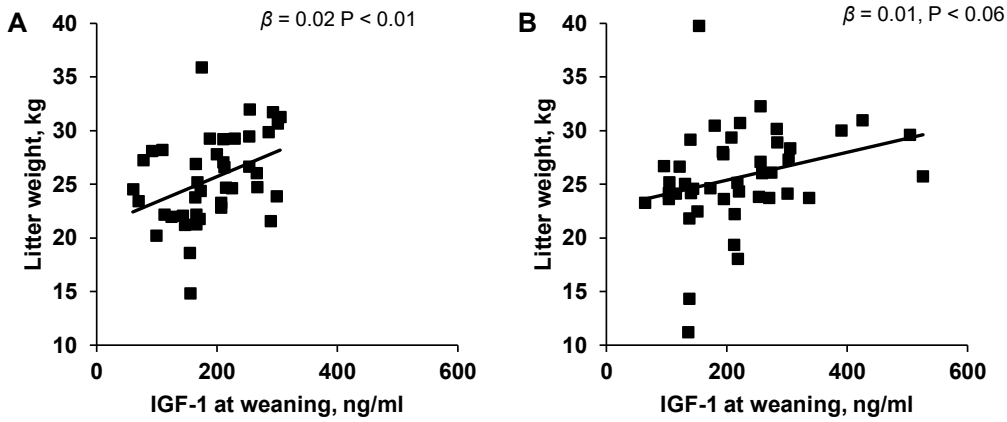


Fig. 5.6. Regression equations (β) for the relations between IGF-1 concentration at weaning and litter weight at subsequent farrowing in sows after their first lactation in Study I (A) and Study II (B).

In Study II, higher IGF-1 concentration at treatment allocation was related to larger mean piglet birth weight at subsequent farrowing (β = 0.6 g per ng/ml, P < 0.04; Fig. 5.7A). However, in Study I, IGF-1 concentration at 3 days after weaning in sows after their first lactation was positively related to mean birth weight at subsequent farrowing (β = 1.1 g per ng/ml, P <0.02; Fig. 5.7B). In both first- and second-parity sows, higher IGF-1 concentrations at oestrus were related to a higher

mean piglet birth weight in Study I (β = 1.0 g per ng/ml, P = 0.03), whereas IGF-1 concentration during WEI was not related to mean piglet birth in Study II.

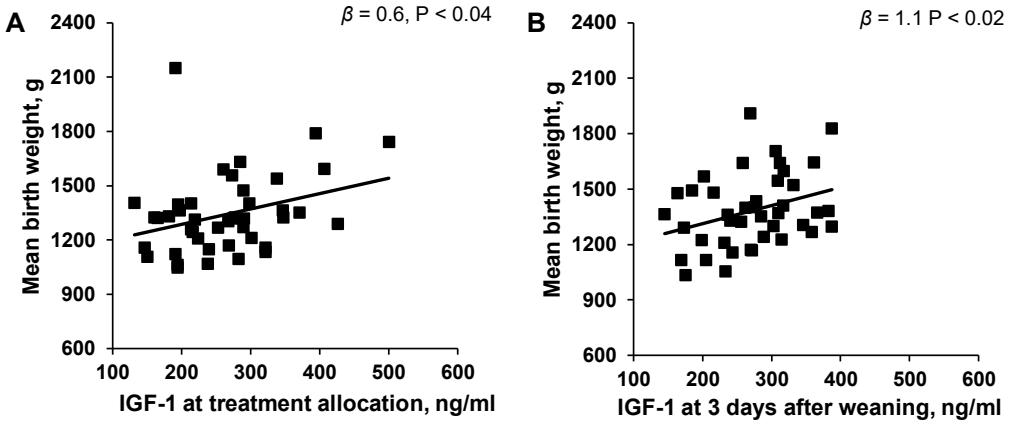


Fig. 5.7. Regression equations (β) for the relations between IGF-1 concentration at treatment allocation and mean piglet birth weight at subsequent farrowing in sows after first lactation in Study II (A) and the relations between IGF-1 concentration at 3 days after weaning and mean piglet birth weight at subsequent farrowing in sows after first lactation in Study I (B).

In Study II, IGF-1 concentration at treatment allocation, i.e. at 1 week before weaning, was negatively related to proportions of piglets < 1 000 g (β = -0.04% per ng/ml, P < 0.02; Fig. 5.7A). In Study I, higher IGF-1 concentration at oestrus after first lactation was related to lower proportions of small piglets at subsequent farrowing (β = -0.07% per ng/ml, P < 0.01; Fig. 5.8B).

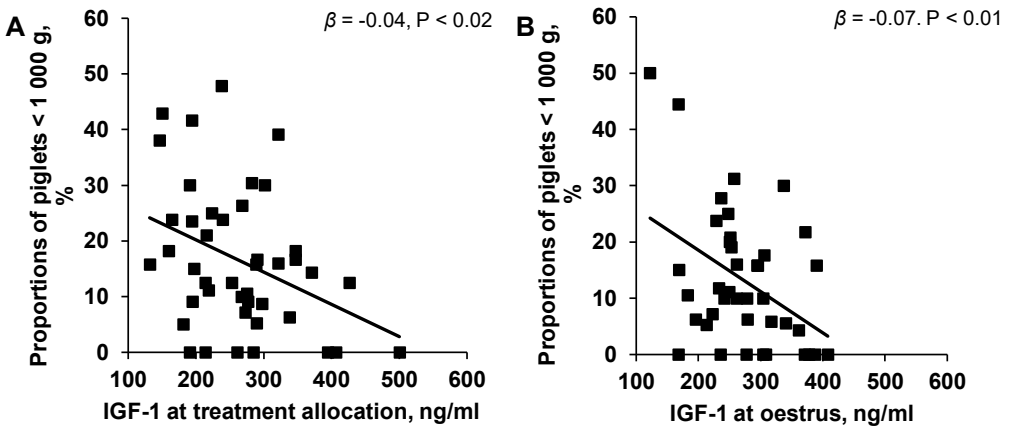


Fig. 5.8. Regression equations (β) for the relations between IGF-1 at treatment allocation and proportions of piglets < 1 000 g at subsequent farrowing in sows after first lactating in Study II (A) and relations between IGF-1 at oestrus and proportions of piglets < 1 000 g at subsequent farrowing in sows after first lactating in Study I (B).

In Study I, no interactions between parity and body condition loss characteristics were observed (P > 0.05). Body weight loss and BF loss were not related to any litter characteristics in either Study I or Study II. However in Study I, higher LM loss during the previous lactation was related to lower CV of birth weight at subsequent farrowing (β = −0.1%/%, P < 0.05; Fig. 5.9A). Further, in Study II, higher LM loss during the last week of previous lactation was related to higher proportions of

piglets < 1 000 g (β = 1.1%/mm, P < 0.05; Fig. 5.9B) and lower litter weight (β = -0.5 kg/mm, P < 0.01) at subsequent farrowing. When corrected for the total number of piglets born per litter, LM loss during the last week of the previous lactation was negatively related to mean piglet birth weight at subsequent farrowing (β = -13 g/mm, P = 0.02).

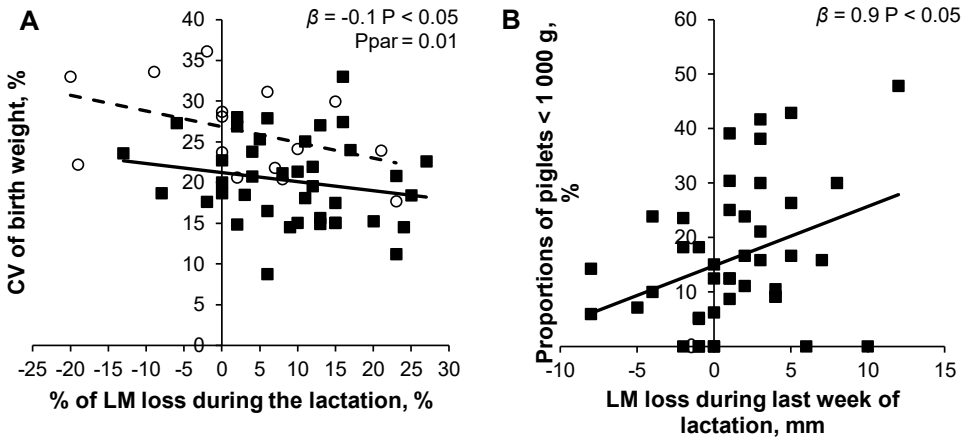


Fig. 5.9. Regression equations (β) for the relations between loin muscle depth (LM) loss during previous lactation and CV of birth weight (A) at subsequent farrowing in sows after their first (■, solid line) and second lactation (○, dotted line) in Study I and relations between LM loss during the last week of previous lactation (1 week before weaning) and proportions of small piglets in Study II (B).

In Study II, NEFA concentration at weaning was negatively related to the total number of piglets born per litter (β = -0.05 n per µmol/L and β = -3.1 n per mmol/L, P < 0.05 for both). Also, creatinine concentration at weaning was negatively related to litter weight (β = -0.10 kg per µmol/L, P < 0.02; Fig. 5.10A). Higher NEFA concentration at oestrus was related to higher CV of birth weight (β = 28.8% per mmol/L, P = 0.05; Fig. 5.10B).

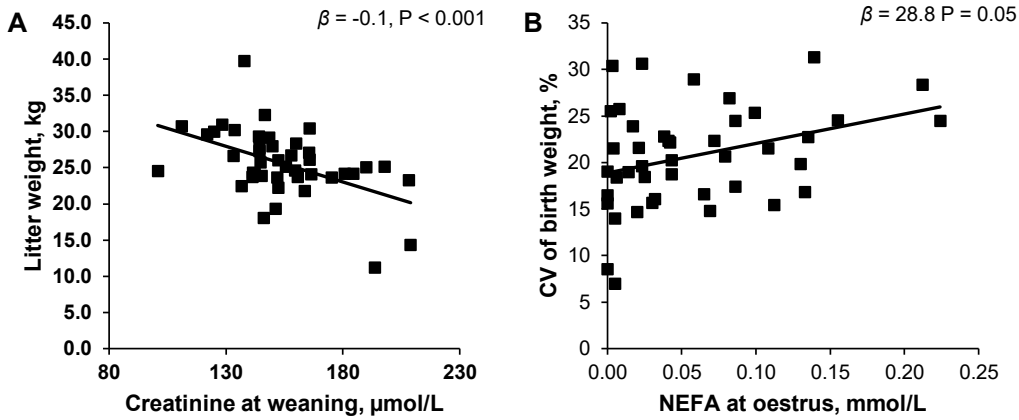


Fig. 5.10. Regression equations (β) for the relations between serum creatinine concentration at weaning and litter weight (A), and the relations between serum NEFA concentration at oestrus and CV of birth weight (B) in first-parity sows in Study II.

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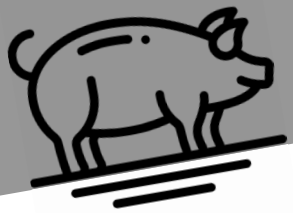

6. DISCUSSION

This study is the first to investigate the impact of pre-mating metabolic state on both follicle development after weaning and piglet birth weight at subsequent farrowing in young sows. Amino acids (AA) supplemented pre-mating diet did not alter the sows' pre-mating IGF-1, while a dietary fibre (DF) supplemented pre-mating diet affected body condition losses during lactation and metabolic hormones after weaning. However, supplementation of both AA and DF to the pre-mating diet did not affect follicle development or piglet birth weight. Although the pre-mating diets had no effect on follicle development or on piglet birth weight, we did find that metabolic hormones, related to body condition losses during lactation, affected both follicle development and piglet birth weight. These relationships differed between the two studies, which may be related to the different levels of NEB during lactation in both studies (12.0% and 7.8%, respectively for Study I and II). This discussion will explore the possible explanations of these findings, focusing on consequences of the pre-mating diets, body mobilisation patterns and the impact of IGF-1 and other metabolic hormones on follicle development and piglet birth weight as summarized in Fig. 6.1.

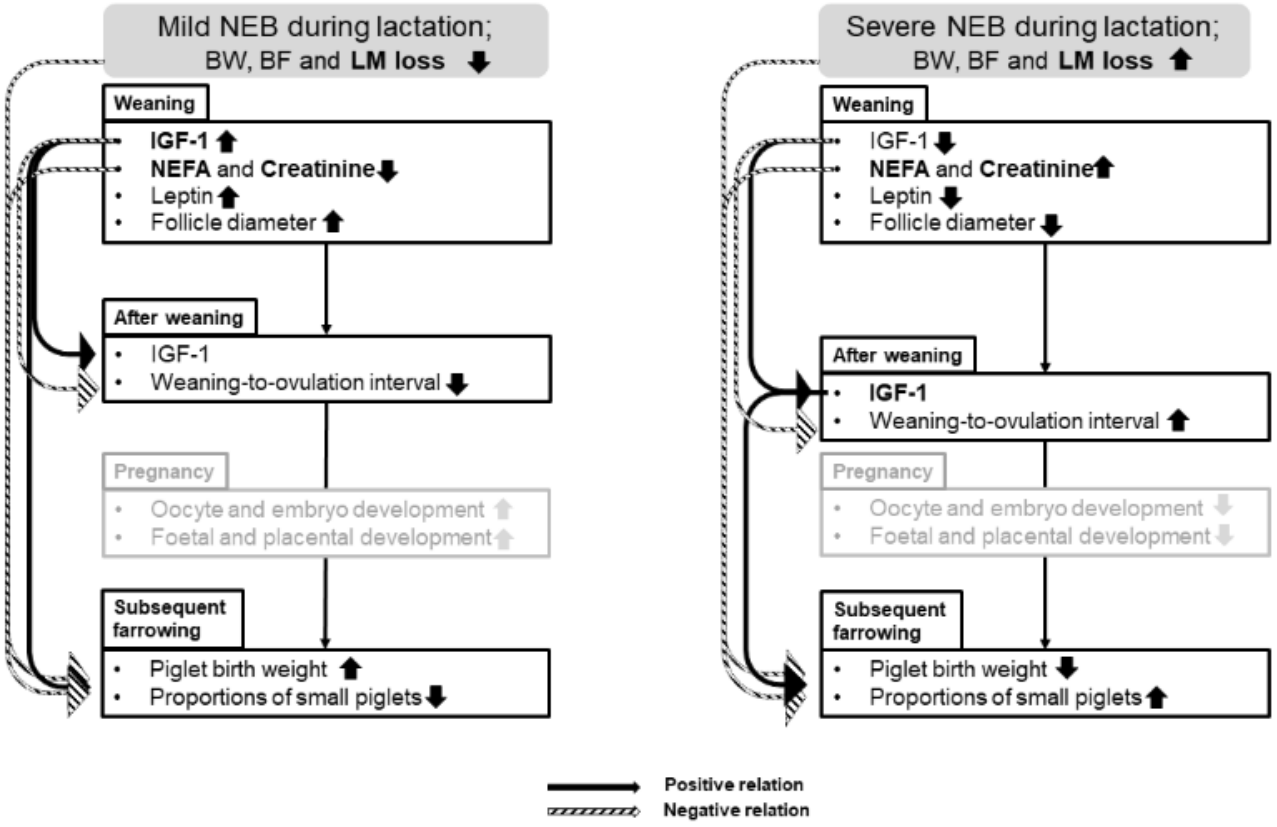

Fig. 6.1. Schematic overview of 1) body condition losses, metabolic hormones, follicle development and litter characteristics at subsequent farrowing and 2) relationships between pre-mating metabolic state, follicle development and litter characteristics in sows with mild and severe negative energy balance (NEB) during lactation in this thesis. Bold letters are the pre-meting metabolic state which is directly associated with piglet birth weight at subsequent farrowing. Up and down arrows indicate the comparison between sows with mild and severe NEB during lactation.

6.1. Pre-mating diets and pre-mating metabolic state

Pre-mating diets known for increasing IGF-1 concentration after feeding (e.g. AR and LC, see paragraph 2.5) did not alter the IGF-1 concentrations during WEI (Study I). Even though we supplemented at a similar or higher level than that known to increase IGF-1 during gestation and lactation (1 or 1.5% of AR of total feed amount and higher than 0.23 kg/day of LC in this thesis), pre-mating diets failed to modulate IGF-1 concentration during WEI. The lack of effect of pre-mating diets on IGF-1 concentration might be related to the NEB of sows during lactation and sows' rapid recovery of IGF-1 concentration after weaning. After weaning, sows change toward an anabolic state, which involves rapid restoration of plasma IGF-1 concentrations (see paragraph 2.4). This restoration of IGF-1 seems to be affected by the level of NEB during lactation. Sows in Study I, who experienced mostly severe NEB, had a rapid recovery of IGF-1 levels within 3 days after weaning (on average + 71 ng/ml during first three days after weaning), and no relationships between body condition losses and IGF-1 concentration were observed after 3 days after weaning. However, sows in Study II, who experienced mostly mild NEB, had a lower increment of IGF-1 than sows in Study I. Similarly, in another recent study of ours (Han et al., in preparation), sows fed a full-fed during lactation (4.0% of BW loss during lactation) had no increase of IGF-1 concentrations between weaning and 3 days after weaning, whereas sows restricted-fed (18.2% of BW loss during lactation) had high IGF-1 increment of 112 ng/ml from weaning to 3 days after weaning. It seems that sows with severe NEB have a higher ability to restore IGF-1 after weaning (Lucy, 2008). This increment in IGF-1 concentrations after weaning in sows with severe NEB resulted in a different IGF-1 pattern during WEI. In study I, plasma IGF-1 was low at weaning and increased towards oestrus. In Study II, IGF-1 concentration was high at weaning and constant until oestrus. This NEB-based difference in the IGF-1 concentration pattern may also be the reason for the different relationships between IGF-1 and piglet birth weight at subsequent farrowing. Thus, sows' NEB has a major impact on IGF-1 concentration patterns after weaning, which attenuate the effect of pre-mating diets on these patterns.

The role of growth hormone (GH) on IGF-1 during anabolic state (after weaning) is a possible reason for the rapid increment of IGF-1 in sows with severe NEB. Sows with severe NEB during lactation had higher GH and lower IGF-1 the day before weaning (Mejia-Guadarrama et al., 2002). This is because higher GH facilitated the mobilization of lean tissue, and uncoupling between GH

and IGF-1 resulted in lower IGF-1 during lactation (Mejia-Guadarrama et al., 2002; Lucy, 2008; see paragraph 2.4). This higher GH before weaning may stimulate the increment of IGF-1 after weaning via hepatic response. In growing-finishing pigs, protein restriction resulted in higher GH and lower IGF-1 than control pigs (Ju et al., 2021; Zhang et al., 2021), similar to sows with severe NEB. During the compensatory growth period (anabolic state), protein-restricted pigs had lower serum GH and higher IGF-than control pigs (Ju et al., 2021; Zhang et al., 2021). However, protein-restricted pigs had increased GH receptor and IGF-1 mRNA expression in the liver (Ju et al., 2021; Zhang et al., 2021). This increased GH receptor may stimulate IGF-1 production in the liver during compensatory growth because GH molecules binding to GH receptor promote IGF-1 production in the liver (Zhang et al., 2013). Similarly, GH also stimulates higher IGF-1 secretion during anabolic state in sows who have increased expression of GH receptor. Thus, it seems that increased GH receptor and IGF-1 mRNA in the liver may be one of the reasons for the rapid restoration of IGF-1 concentration after weaning. However, no study has investigated the impact of NEB on GH and IGF-1 secretion together with receptor expression in the liver in sows.

Pre-mating diets did not modulate follicle development and subsequent fertility similar to IGF-1. In sows fed DF, we expected to find a lower E2 concentration before ovulation and improved oocyte and embryo development (by larger follicle and piglet birth weight) similar to what Ferguson et al. (2007) found in gilts, but we failed to find an effect. Gilts usually don't experience NEB before mating. Also, gilts used in the study of Ferguson et al. (2007) were fed DF supplemented pre-mating diets starting at the second day of oestrus, which implies that those gilts are in a similar follicular developmental stage. However, sows who experienced NEB during lactation have a huge variation of follicle developmental at weaning and thereby have different follicle development after weaning. We also found that the level of NEB during lactation resulted in a huge variation of follicle development (2.7-4.7 mm at weaning) and time of ovulation (i.e. WOI: 101-185 h). Sows' BW at weaning was more homogeneous (i.e. less variation; SEM = 2) in the study of van den Brand et al. (2009), who found the positive impact of sugar-rich pre-mating diets on piglet birth weight, compared to our study (SEM = 5). Although follicle development was not investigated in the study of van den Brand et al. (2009), homogenous BW at weaning may be connected to less variation in follicle development within sows. Thus, the effects of pre-mating diets on reproductive parameters seem to be more easily detected if there is less variation of follicle development. Further, the positive impacts of pre-mating diets in the study of van den Brand et al. (2009) may

be also due to the advanced follicle development after weaning because of less body condition losses. Body weight loss during lactation in the study of van den Brand et al. (2009) was on average 4.1%, which is much lower than that of Study II (7.8%). Similarly, the oestrus rate in the study of van den Brand et al. (2009) was much higher compared to our studies (98 v. 82 and 86%). Thus, a homogeneous follicle developmental stage with more advanced follicle development (i.e. not compromised) after weaning within sows may be required to find the effects of pre-mating diets on reproductive parameters and subsequent piglet birth weight.

The higher BF loss in SBP sows during 1 week before weaning can be partly explained by their reduced feed intake (6.20 v. 5.69 v. 6.61 kg/day for CON, SBP, MFC, respectively, $P < 0.07$; Study II). Presumably, SBP reduces gastrointestinal flow of digesta (Bach Knudsen, 2001) in first-parity sows with lower gastric capacity compared to multiparous sows (Theil et al., 2012) can explain lower feed intake during late lactation. Thus, supplementation with a high amount of SBP may reduce feed intake during lactation, especially in young sows. Surprisingly, the higher BF loss did not result in a higher NEFA concentration at weaning in SBP sows, although they did have a higher creatinine concentration at weaning. Likewise, in first-parity sows, Costermans et al. (2020a) found different NEFA concentrations at weaning between sows that were full-fed and restricted fed during the last 2 weeks of lactation, but creatinine concentration was higher in the restricted-fed sows. Contrary to our finding, BF loss and LM loss were negatively related to NEFA and creatinine (Costermans et al., 2020a). Possibly, the body reserve and its mobilization pattern differed between the breeds used (DanAvl v. TN70, this thesis and Costermans et al., 2020a). In addition, NEFA concentration at weaning in SBP sows was higher than in MFC sows (0.523 v. 0.365, $P = 0.12$, Study II). Thus, it seems that lower feed intake in SBP sows results in higher body mobilization during lactation than in MFC sows. Nevertheless, reproductive parameters and litter characteristics at subsequent farrowing were similar in SBP and MFC sows.

6.2. Pre-mating metabolic state, follicle development and piglet birth weight

Although we failed to modulate the pre-mating state by supplementing pre-mating diets, our findings support the second hypothesis of this thesis that pre-mating metabolic state is related to follicle development and piglet birth weight. Moreover, a pre-mating metabolic state connected to lower body condition losses (higher IGF-1, and lower NEFA and creatinine) was related to a higher average piglet birth weight. Also, pre-mating IGF-1 concentrations were related to both post-weaning follicle development and subsequent piglet birth weight. The impacts of pre-mating IGF-1 on follicle development and thereby piglet birth weight seem to depend on sow's NEB during lactation. We found that pre-weaning IGF-1 has a beneficial impact on piglet birth weight in sows with mild NEB during lactation (Study II). Further, IGF-1 concentrations after weaning was positively related to piglet birth weight in sows with severe NEB (Study I). When we classified the first-parity sows into severe and mild NEB (BW ≥ 10% and BW < 10%, respectively), similar relationships were observed; pre-weaning (i.e. one week before weaning) IGF-1 positively affected piglet birth weight in sows with mild NEB, whereas IGF-1 during the WEI had a beneficial impact on piglet birth weight in sows with severe NEB (Fig. 6.2). Thus, it seems clear that the relationships between pre-mating IGF-1 and piglet birth weight depend on the level of NEB during lactation in young sows.

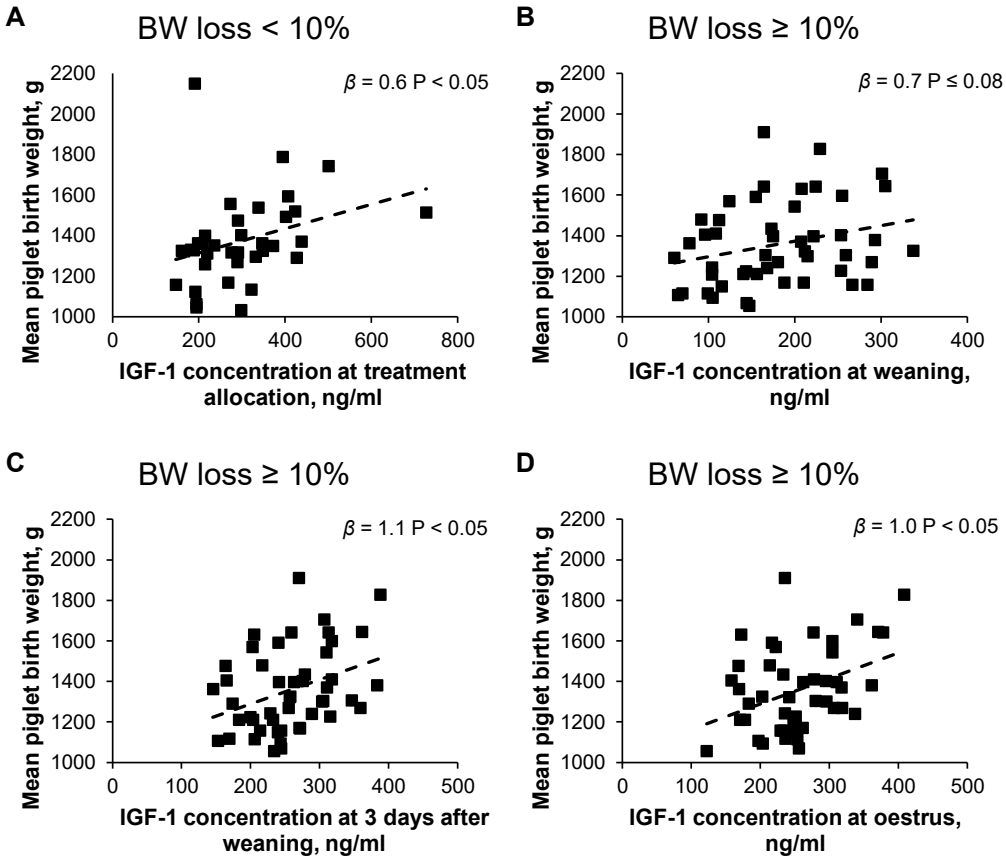

Fig. 6.2. Regression equations (β) for the relations between mean birth weight at subsequent farrowing and IGF-1 concentration at treatment allocation (1 week before weaning) in sows with BW loss < 10% during the lactation in both studies (A), and relations between mean birth weight and IGF-1 during WEI (at weaning (B), at 3 days after weaning (C) and at oestrus (D)) in sows with BW loss ≥ 10% during first lactation in both studies.

The different relationships between IGF-1 and piglet birth weight based on the level of NEB during lactation may be due to the different role of IGF-1 on follicle development before ovulation. It seems that higher IGF-1 before weaning may affect follicle recruitment and selection around weaning, and thus oocyte quality may be affected by IGF-1 in sows with mild NEB (also in Study II). However, if sows had lower plasma IGF-1 concentrations at weaning due to severe NEB (also in Study I), plasma IGF-1 concentration may be too low to stimulate follicle and oocyte development right after weaning. However, when IGF-1 secretion is recovered around 3 days after weaning, plasma IGF-1 may affect follicle and oocyte development. Later on, follicle and oocyte development associated with plasma IGF-1 after recovery would reflect in the piglet birth weight. Our recent study (Han et al., in preparation) also indicates that plasma IGF-1 is related to follicular fluid IGF-1 after recovery of plasma IGF-1 in sows with severe NEB. We found that sows under feed restriction during the last 2 weeks of lactation (severe NEB) had less developed follicles at 4 days after weaning compared to sows fed full-fed during lactation (mild NEB; Han et al., in preparation). At this time, the feed restricted sows had smaller follicles with higher oestrogen and lower progesterone concentrations compared to the well fed sows (Han et al., in preparation). This steroid profiles indicate that feed restricted sows have less developed follicle pools around the time of oestrus (i.e. 4 days after weaning), whereas more developed follicle pools (i.e. luteinizing follicle) are observed in well fed sows based on the steroids profiles (Han et al., in preparation). This is because pre-ovulatory estrogenic follicles are observed during the time of oestrus, while pre-ovulatory luteinized follicles are found at 2 days of oestrus (Agca et al., 2006). Also, plasma IGF-1 concentration at 3 days after weaning was strongly related to follicular fluid IGF-1 at 4 days after weaning in restricted fed sows (i.e. compromised follicle pool; $\beta = 0.89$, $P < 0.05$). This relationship was not found in full-fed sows (i.e. normal follicle pool; Han et al., in preparation). Thus, especially in sows with severe NEB, it seems that plasma IGF-1 at the late follicular phase (i.e. 3 days after weaning) affects follicle and oocyte development and thereby affects subsequent fertility.

In sows with mild NEB, on the other hand, follicle development seems to be affected by IGF-1 already at the time of weaning (or before weaning), and the impact of IGF-1 on follicle development after weaning may be attenuated. One experiment supplementing exogenous IGF-1 in follicular fluid from large follicles (6-10 mm) during IVM showed that exogenous IGF-1 negatively affect oocyte maturation (Oberlender et al., 2013). Although, without exogenous IGF-1,

oocytes maturation rate in follicular fluid from large follicles was higher than that in follicular fluid from small follicles (2-5 mm; Oberlender et al., 2013). The increased supplementation level of IGF-1 in follicular fluid (from 0 to 240 ng/ml) in large follicles was negatively related to oocytes maturation rate (β = -0.05% per ng/ml, $P < 0.01$; Oberlender et al., 2013). This is because, in large follicles, there may be desensitization of IGF-1 receptor by excessive presence of the IGF-1 (reviewed by Oberlender et al., 2013) due to high concentrations of IGF-1 in follicular fluid from large follicles (Liu et al., 2000; Costermans et al., 2020a). Moreover, the abundance of IGF binding protein (IGFBP) in follicular fluid of large follicles resulted in a smaller impact of IGF-1 on oocyte quality (Demeestere et al., 2004). Thus, we can speculate that follicles are already positively affected by plasma and follicular fluid IGF-1 in sows with higher IGF-1 at weaning (sows with mild NEB). Similar to our finding, supplementation of sugar source during the whole lactation positively affected piglet birth weight in sows with mild NEB (4.1% of BW loss) as discussed above (van den Brand et al., 2006 and 2009). Probably, supplementation of sugars increased plasma IGF-1 during lactation (i.e. pre-weaning; not investigated), which has a beneficial impact on piglet birth weight in sows with mild NEB. Nevertheless, the investigation of the different effects of IGF-1 in sows at different levels of NEB (severe or mild) are required.

Further, our findings indicate that higher plasma IGF-1 during lactation is beneficial for piglet birth weight at subsequent farrowing; higher plasma IGF-1 concentrations are related to larger mean piglet birth weight and lower proportions of small piglets. We observed that IGF-1 concentrations before weaning were highly correlated to those during WEI in both studies, as also shown previously (van den Brand et al., 2001; Wientjes et al., 2012b and 2013a). Thus, higher concentrations of IGF-1 during lactation positively affect piglet birth weight in sows with mild NEB. On the other hand, if sows with severe NEB, higher IGF-1 during lactation is connected to that during WEI, which also positively affect piglet birth weight. Thus, minimizing sows' body condition losses is an option for increasing piglet birth weight and decreasing proportions of small piglets.

Also other metabolic hormones were related to follicle diameters after weaning and piglet birth weight, indicating that minimizing body mobilization is important for subsequent fertility. We found a tendency for a negative relationship between NEFA and follicle diameter (data not shown), which was also observed in first-parity sows in another study (Quesnel et al., 1998b). Surprisingly, in multiparous sows, Costermans et al. (2019b) found a tendency for a positive correlation between follicle diameter at weaning and NEFA concentrations, whereas creatinine

concentration at weaning was negatively related to follicle diameter. The different relationships between NEFA and follicle development in first-parity versus multiparous sows may be related to a different negative energy balance during lactation. Therefore, we can speculate that higher body lipid mobilization after weaning may negatively affect oocyte development in younger sows. We also found that sows with higher NEFA after weaning had higher variation of piglet birth weight at subsequent farrowing. Also, NEFA concentration at oestrus tended to be negatively related to piglet mean birth weight ($P = 0.07$, data not shown). Thus, piglet birth weight, which is affected by follicle and oocyte development, may be affected by body fat mobilization during lactation. The positive relationship between leptin concentrations at weaning and follicle diameter at weaning is similar to Costermans et al. (2020a). They found a similar positive relationship at 2 days after weaning in full-fed first-parity sows but no relationship was found in restricted-fed sows. This implies that first-parity sows' body fat mass has a beneficial impact on follicle and oocyte quality. Again, better sows' metabolic state during lactation and WEI (i.e. higher IGF-1 and leptin, and lower NEFA, creatinine and urea) are essential for follicle development and subsequent piglet birth weight.

Loin muscle depth loss during lactation had higher impact on IGF-1 at weaning than BW loss and BF loss in Study I. This implies that sows' LM loss has a greater effect on subsequent fertility than BW or BF. Previous studies also report that LM can be a major measurement during lactation. For instance, sows restricted-fed during the last 2 weeks of lactation had higher LM loss and lower follicle diameters after weaning, while BF loss was similar to that of full-fed first-parity sows (Costermans et al., 2020a). Further, first-parity sows with high BW loss had higher LM loss during lactation and lower embryo survival during early pregnancy, while BF loss was comparable to sows with low lactation weight loss (Hoving et al., 2012). Our findings support the impact of LM loss on follicle and embryo development as we found this impact on litter characteristics in both studies. Therefore, although measuring LM in a practical situation is not easy, we suggest that LM loss during lactation represents better for sows' body mobilization and its impact on subsequent fertility.

We found, however, somewhat contrasting results for the impact of LM loss on litter characteristics between the two studies. In Study 1, lactation LM loss positively affected SD and CV of birth weight at subsequent farrowing in sows of both parity. In Study II, higher LM loss during late lactation was not related to SD and CV of birth weight. However, higher LM loss during late

lactation negatively affected litter weight, mean birth weight and increased proportions of small piglets at subsequent farrowing. This discrepancy in the effects of LM loss on litter characteristics between the two studies may be related to the different levels of NEB during lactation between the studies. The higher proportions of small piglets in sows with higher LM loss during late lactation in Study II seem to correspond to the finding of Wientjes et al. (2013b), who showed that sows with higher BW loss and BF loss had a large variation of piglet birth weight (SD and CV of birth weight) in mixed parity sows (on average 3.2). This indicates that high body condition losses negatively impact subsequent fertility, as we hypothesized. However, BW loss and BF loss during lactation were not related to piglet birth weight in this thesis. This may be because LM loss has more impact on reproductive performance in young sows compared to BW and BF. Our finding that IGF-1 at weaning is only affected by LM loss during lactation (Study I) may support this speculation.

In contrast to the findings in Study II, the higher LM loss during lactation was related to less variation in piglet birth weight in Study I. This was surprising since we expected that a more severe NEB (as found in Study I) would be related to impaired litter characteristics, such as low birth weight or larger variation in birth weight. One possible explanation for the increased litter uniformity in sows with increased LM loss in Study I might be a reduced ovulation rate and thus fewer embryos in sows with higher LM loss during lactation. In both studies, sows with a higher LM loss during lactation had a reduced follicle size at weaning and a longer WOI. Longer WOI is connected to fewer LH pulses and lower ovulation rate (van den Brand et al., 2000; Bracken et al., 2003). As the number of ovulations decrease, fewer and more uniform embryos may attach to the uterus (Da Silva et al., 2017b). Also, Hoving et al. (2012) found that sows with higher LM loss during lactation had lower numbers of embryos and implantation sites and reduced embryo survival on day 35 of pregnancy. Both reduced ovulation rate and reduced number of implantations may result in better litter uniformity.

We investigated the impact of the level of NEB to further clarify relations between lactation LM loss and subsequent litter characteristics. In sows with a severe NEB during lactation (BW loss ≥ 10%), LM loss during lactation was not related to any of the litter characteristics (results not shown), which is not in the same line with Study I. Higher LM loss during late lactation in mild NEB (BW loss < 10%) was related to lower mean birth weight and lower proportions of big piglets (results not shown), which is similar to Study II. Thus, a different level of NEB during lactation does

not seem to be a cause of the different relationships between lactation LM loss and litter characteristics between the two studies. However, we speculate that protein mobilization during late lactation partly (or at least in this thesis) affects piglet birth weight characteristics because we found that creatinine concentration at weaning was negatively related to litter size. Thus, proper lactation feeding strategies, especially for body protein achieved by optimized protein and AA content, are recommended for improving piglet birth weight. In addition, more studies are needed about sows' LM loss during lactation and their relationship between body protein mobilizations.

In addition to our findings on the importance of pre-mating nutrition and metabolic state, there are also other approaches for increasing piglet birth weight and decreasing within-litter piglet birth weight variation (i.e. improving litter uniformity). Numerous studies suggested that genetic selection for piglet birth weight and litter uniformity are interesting because of their high maternal heritability (Daamgard et al., 2003; Kapell et al., 2011). Besides, it has been becoming more feasible to investigate genes associated with better follicle and oocyte development and their heterogeneity thanks to advanced technologies such as RNA sequencing. Similarly, genome-wide association studies (GWAS) provided interesting genes from blood related to litter uniformity in sows as a marker for genetic selection (Wang et al., 2017). Therefore, we recommend that both optimizing sows' pre-mating state and genetic selection focusing on piglet birth weight and its variation is possible options for improving farm productivity and animal welfare issue in large litters.

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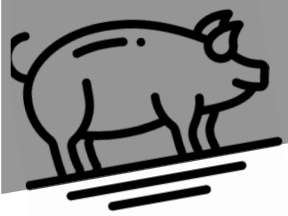

7. CONCLUSIONS, PRACTICAL IMPLICATIONS AND FUTURE RESEARCH

7.1. Conclusions

1. Supplementation of amino acids (AA; L-carnitine (LC) and L-arginine (AR)) in pre-mating diets (during one week before weaning plus WEI) did not affect IGF-1 concentration, probably due to IGF-1 restoration after weaning. However, dietary fibre (DF; microfibrillated cellulose (MFC) and sugar beet pulp (SBP)) supplemented pre-mating diets differently affected feed intake and body mobilization during late lactation.

2. Both AA and DF supplemented pre-mating diets did not affect follicle development and reproductive parameters. This is because sows NEB during lactation, which is largely affected by feed intake during lactation, had a larger impact compared to pre-mating diets on follicle development and subsequent reproductive parameters.

3. Piglet birth weight was not affected by AA and DF supplemented pre-mating diets. This is also because sows' NEB during lactation had a larger effect compared to pre-mating diets on sows' follicle development and subsequent piglet birth weight.

4. Higher body mobilization during lactation was related to smaller follicle diameters after weaning. This is mediated by the impact of pre-mating metabolic state (IGF-1, NEFA, creatinine and leptin) on follicle development.

5. Pre-mating metabolic hormones (IGF-1, NEFA and creatinine) affect both follicle development and litter characteristics. However, the impact of IGF-1 on litter characteristics (possibly via follicle development) depends on the level of NEB during lactation. Nevertheless, our finding suggested that such strategies as optimized lactation diets and management during lactation are needed for increasing piglet birth weight in large litters.

7.2. Practical implications

1. Higher feed intake during lactation with optimized lactation diets is recommended for sows with large litters in order to have larger piglet birth weight and fewer small piglets at subsequent farrowing.

2. If sows have longer weaning-to-ovulation interval (also can be recognized as prolonged weaning-to-oestrus interval by farmers) with skinny body condition (i.e. after losing a large amount of body condition during lactation), there is a high chance to have lower birth weight piglets and higher proportions of small piglets.

3. Sows with severe NEB during lactation will need additional management at subsequent farrowing, especially in order to help them to care for low birth weight piglets.

4. Use of ultrasonography to measure loin muscle depth during lactation and follicle diameters during the weaning-to-oestrus interval are recommended for a better understanding of sow reproduction and their productivity.

7.3. Future research

1. Optimization of lactation diet in hyper-prolific sows.
    - ✓ This can be done by investigating the optimal energy or protein requirements during lactation for milk production and follicle development. Not only current reproduction (i.e. lactation and piglet growth) but also subsequent reproduction cycle (i.e. follicle development after weaning and piglet birth weight at subsequent farrowing) should be considered. Also investigation of energy requirements and utilization during WEI is needed.

2. Utilization of plasma IGF-1 on follicle development based on different level of NEB during lactation.
    - ✓ Investigation of the impact of plasma and follicular fluid IGF-1 on follicle and embryo development is needed as plasma IGF-1 differently affect piglet birth weight. Different levels of NEB should be considered in this research. To this end, feed restriction during late lactation for intentional severe NEB can be applied.

3. The specific mechanism of rapid IGF-1 restoration in sows with severe NEB during lactation.
    - ✓ Growth hormone or other IGF-1 binding proteins may contribute during the process of rapid IGF-1 restoration. Thus, associations between GH and IGF-1 after weaning (anabolic state) until ovulation should be studied. Growth hormone receptor, IGF-1 and IGF-1 receptor expression in the liver may be used to elucidate the reason for the rapid restoration of IGF-1 after weaning in sows with severe NEB.

4. Metabolic hormone profiles between sows' blood and follicular fluid in pre-ovulatory follicles.
    - ✓ Follicular fluid is an exudate of blood, blood metabolic hormones and amino acids represent follicular fluid, which is the microenvironment for follicle and oocyte development. The different hormone and proteomic profiles between sows' blood and follicular fluid may provide new insights into the nutrient requirements for follicle development after weaning. This will be useful for optimizing a lactation diet and even a diet during WEI (flushing).

5. Sows' BW, BF and LM during lactation and their relationships with body chemical compositions and milk production.
    - ✓ Earlier studies have supplied an equation for predicting sows' milk production based on sows' BW and BF. However, LM should be added to those equations as our study showed that LM can also be measured and represents sows' body condition. This may provide a more accurate equation for predicting sows' body protein and lipid reserves.

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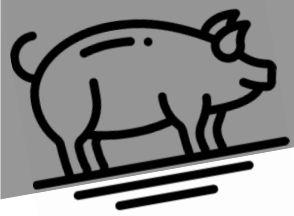

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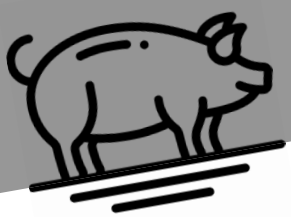
9



9. ORIGINAL ARTICLES